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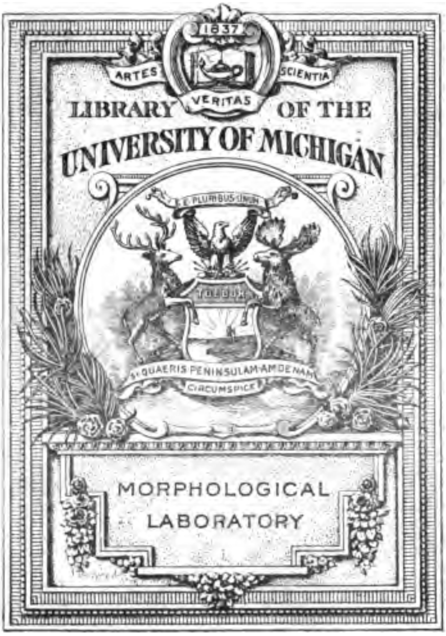

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THE BREEDING HABITS
AND THE
SEGMENTATION OF THE EGG
OF THE
PIPEFISH
(*SIPHOSTOMA FLORIDÆ*)

BY

EUGENE WILLIS GUDGER

Dissertation submitted to the Board of University Studies of the Johns Hopkins University in conformity with the requirements for the Degree of Doctor of Philosophy: Baltimore, 1905

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THE BREEDING HABITS AND THE SEGMENTATION OF THE EGG OF THE PIPEFISH, SIPHOSTOMA FLORIDÆ.

By EUGENE WILLIS GUDGER,

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INTRODUCTION.

Through the kindness of Prof. W. K. Brooks, it was made possible for me to go to Beaufort, North Carolina, in the summer of 1902, and while there I began, at his suggestion, to collect material for the development of the head skeleton of the pipefish. I soon found young embryos and segmenting eggs, and, wishing to take up the embryology of this fish, I deferred the former work till a later date.

The collecting of further material and the observations on the breeding habits were made at Beaufort during the summers of 1903 and 1904, when, with running sea water at hand, the difficulties necessarily attendant on this work were materially reduced.

This preliminary work was done in the laboratory of the United States Bureau of Fisheries at Beaufort, North Carolina. I am indebted to the Commissioner, Hon. George M. Bowers, for the opportunity to make use of the most excellent facilities at hand there. To the director, Dr. Caswell Grave, I am under obligations for many helpful suggestions.

The further work was done in the biological laboratory of the Johns Hopkins University. To Prof. W. K. Brooks, I am very grateful for the interest taken in my work and for advice and direction. I also wish to thank Dr. E. A. Andrews and Dr. Caswell Grave for advice in overcoming the technical difficulties of my work.

MATERIAL AND METHODS.

Male pipefishes with full pouches were brought into the laboratory, and there the upper end of the pouch was opened with forceps and a few eggs removed and put under the microscope. If these were in a stage wanted, the head of the fish was cut off, the flaps of the pouch slit open with scissors and removed (frequently bringing eggs with them), and the eggs removed by tearing with needles the tissue binding them down. If the eggs were too young, the fish was put back in running water and examined again later, although it rarely survived a second operation unless the eggs were newly laid and hence came out easily. This is a wasteful process, since many eggs are spoiled in

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removing them. The obtaining of a series of eggs and embryos of *Siphostoma* is a long, slow, and laborious task and is quite as much the result of chance as of skill and knowledge.

A variety of killing fluids has been used. The oil drops under the germ disk were so blackened by osmic acid and Flemming's fluid that these reagents could not be used. Acetic alcohol, Kleinenberg, sublimate-acetic, picro-acetic, all gave good blastoderms, but the yolks generally went to pieces. Excellent results were obtained with *fresh* Perenyi, 10 per cent, and 20 per cent formalin, and, for later stages, Gilson's and Worcester's fluids. This latter is one of the best fluids for killing teleostean eggs with which I am acquainted. It is composed of saturated sublimate in 10 per cent formalin, 90 parts; glacial acetic, 10 parts. The eggs are left in this from thirty to sixty minutes, washed in water, run up into 70 per cent alcohol, and the excess of sublimate removed with iodin.

The eggs, bound up in masses when taken from the watery killing fluids, were sometimes put into a 10 per cent solution of hypochlorite of sodium or potassium to soften the connective tissue and the transparent egg membranes. Over-exposure to these fluids was very hurtful to the blastoderms, and generally the eggs were run up into 70 per cent alcohol and the shells removed with needles.

The younger blastoderms were picked off the yolks and sectioned, but the protoplasmic processes from the periblast made it impracticable to get the blastoderms in late stages away whole. These eggs were cut whole, and for this purpose those killed in Perenyi's fluid, on account of their soft yolks, were especially good. The yolks of eggs killed in formalin, if kept in alcohol long, tend to become hard, hence they should be gotten into paraffin as quickly as possible.

In order to orient whole eggs in the paraffin it is necessary to stain them. By putting them in full strength borax-carmine for from one to two minutes, the embryonic tissues take the stain before the yolks, and there result red blastoderms on yellow yolks.

The eggs were embedded in paraffin, and sections cut from 5 to 10 microns thick and stained either in Mayer's hæmalum or Heidenhain's iron hæmatoxylin. The former gave such beautiful preparations and was so easy to manage that it was almost exclusively used.

HABITAT.

Pipefishes are found in all the warm and temperate oceans of the world, but are not exclusively marine. Day (1865) reports that *Syngnathus argyrostictus* ascends rivers in Cochin China miles above tide limits. Again (1878), he finds that *S. spicifer*, *Ichthyocampus carce*, and three species of *Doryichthys* go up the rivers of India. Duncker (1904) reports *Doryichthys boaja* and *fluviatilis* in the rivers of the Malayan Peninsula. Such are some of many records.

In the harbor at Beaufort, in quiet shallow waters where there are muddy bottoms, forests of *Zostera* abound and in them the pipefishes live. By fishing in these with a fine-meshed seine, they may be caught in considerable numbers.

It may be well to note that the color of these fishes changes with the seaweeds among which they may be found. *S. floridæ* among tufts of muddy eelgrass is dark green, but put into aquaria with *Codium* or *Ulva* it becomes bright green. *S. fuscum* is ordinarily of a muddy brown color, but several specimens caught in a tide pool filled with red seaweed were brick red in color, and from this were thought to be a new species.

THE BREEDING HABITS OF SIPHOSTOMA FLORIDÆ.

The following observations on the breeding habits of *Siphostoma floridæ* were made in the laboratory of the United States Bureau of Fisheries at Beaufort, North Carolina, July 17, 1903. The transfers were witnessed by three other workers. When my account thereof had been written it was submitted to them and their additions were included in this full statement.

A female fish ready to give up eggs may be recognized by her much distended abdomen, due to the presence of ripe eggs in the ovary, but much more by the oviduct protruding—as first noted by Lafont (1871)—and filled with eggs, some of which may escape from time to time. In the nonbreeding male the flaps of skin forming the pouch lie flat in the ventral concavity formed by the outward and downward projecting skin-covered horny plates of mail, but when sexually excited these flaps rise, become thrown into folds and finally unite their edges into the long middle seam, and form the closed pouch.

The act of copulation is preceded by a very curious "*liebesspiel*." The two fishes swim around in the aquarium with their bodies in nearly vertical positions, but with the head and shoulder region sharply bent forward like the letter f. Then they swim slowly past each other, their bodies touching and the male being perhaps more demonstrative. Just before the actual transfer, the male becomes violently excited and demonstrative, shakes his head and anterior body-parts in a corkscrew fashion and with his snout caresses the female on the belly. The female responds to this but does not become so excited. This is repeated several times, the fishes becoming more excited each time they touch each other. Presently, quick as a flash, the sexual embrace takes place and then the fishes separate to begin again in a few minutes.

NOTE.—A review of the literature from the time of Aristotle to the present, on the sexual characters, breeding habits, and embryonic structure of the Lophobranchs, together with certain notes of my own thereon, is included in my paper as published in full in the Proceedings of the United States National Museum, Volume XXIX, No. 1431.

This embrace consists in the fishes intertwining their bodies like two capital letter S's, the one reversed on the other, thus bringing them face to face. Thus they hold their bodies together while the eggs pass from the oviduct into the pouch. Their bodies touch at three places—in the anterior region, just back of the pectorals; in the posterior region, at a point about two-thirds of the way from the anus to the caudal; and at the anal openings. The anal papilla, or the protruding oviduct of the female, is, at the moment of contact of their bodies, thrust into the buttonhole-shaped opening at the anterior end of the marsupium. Some eggs, in number a dozen or more, now pass into the pouch and are presumably fertilized at this moment.

The eggs are now in the anterior end of the pouch and no more can be received until these have been gotten into the posterior end. To bring this about, the male performs some very curious movements. He stands nearly vertically, and, resting his caudal fin and a small part of the tail on the floor of the aquarium, bends backward and forward and twists his body spirally from above downward. This is repeated until the eggs have been moved into the posterior end of the pouch. I do not think that any means other than the above are used to bring this about. The pouch in a "pithed" fish was opened and carmine scattered over its inner surface, but there was no evidence of ciliary action. Sections from pieces of both dorsal and ventral parts of the sac killed in formalin, in Flemming's or in Worcester's fluids, failed to show cilia.

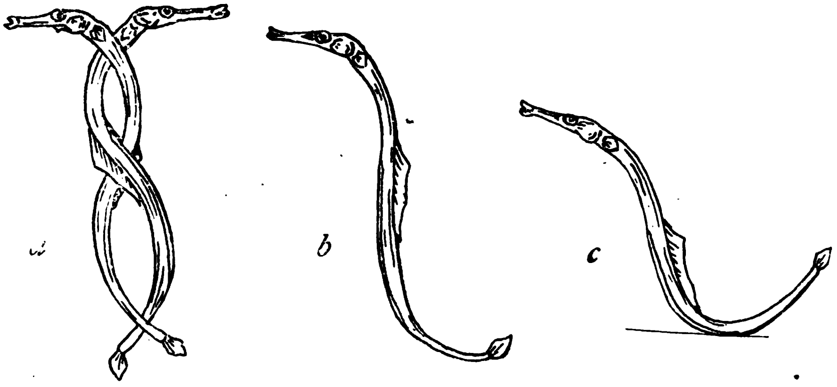

FIG. 1.—TRANSFER OF EGGS IN SIPHOSTOMA (SEMI-DIAGRAMMATIC). *a*, POSITION OF FISHES DURING TRANSFER OF EGGS; *b*, ATTITUDE ASSUMED BY MALE WHILE MOVING THE EGGS BACKWARD IN THE POUCH; *c*, POSITION OF MALE DURING PERIOD OF REST FOLLOWING SEVERAL TRANSFERS.

Then for some time the animals remain quiescent, the male with the back concave, assuming the form of a broad flat capital U. The head is extended in a nearly horizontal direction, and the body in the region of the middle of the tail touches the floor of the aquarium. This position is retained for a time varying from five to ten minutes. Convulsive movements, lasting only for a moment, may take place.

The processes above described are repeated until the pouch is filled. In one pair the first copulation took place at 9.45 o'clock and the second at 10.05 o'clock. In another pair there were four contacts, as follows: 10.15; 10.34; 10.39 o'clock, at which time the eggs were only halfway down the pouch; and at 11.06 o'clock. These observations were made at night, between 9.45 and 11.30 o'clock, in the brightly lighted laboratory. It is very probable, however, that the transfer may take place at any and all hours of the night. It is to be noted in passing that the fishes seemed entirely unaffected by the lights. No attempt to handle them was made. (See Lafont.)

It does not seem likely that all the eggs are transferred at once—first, because of the curious means used to move them backward in the pouch; in the second place, because males are frequently found with the pouch only half filled; thirdly, because males with eggs of two and three stages and layings are not infrequent. When the above processes have been repeated several times, the animals are seemingly exhausted and remain quiet for at least two and one-half hours (the extent of my observations). On this same night a third small male in an aquarium with three females "courted" two of them alternately, but no transfer was made, though they had protruding oviducts. For coition to take place, it seems necessary that the fishes should be nearly equal in size. A ripe female paired with a male three-fifths her size dropped her eggs into the water.

This curious love play above described is not without parallel in other lower vertebrates. Jordan (1891) records for *Diemyctylus* a very interesting series of observations of a courtship, lasting several hours, in which caressings play an important part. Dean (1895), in his account of the spawning of *Lepidosteus*, describes how the males with wide-spread fins swim around the females and caress them with their snouts. Nor is such a courtship unknown among the invertebrates. Racovitza (1894) has described how the male of *Octopus vulgaris* strokes and caresses the female. All these contacts seem to be intended to excite the animals preparatory to the sexual act.

The arrangement of eggs in the pouch depends wholly on the size of the latter. There are always two sets of eggs, one on each side. Each set may consist of one, of two, or of three rows of eggs, and these may be one or two eggs deep. As noted, there may be one, two, or even three deposits of eggs in one pouch. In what order these young would emerge from the pouch I can not say. Ordinarily the seam breaks at points all along its length to set free the young.

The age at time of hatching can be given as ten days (with a variation of eight hours) from one lot only. These young lived four days, feeding on copepods with the same bird-like motion of the head and the same smacking mouth motion found in the parents. In another case, when the father died four days after the transfer, the little fishes were with free tails.

The eggs within twenty-four hours after deposition may easily be extracted from the pouch, coming out in masses, without injuring the father. In two cases, males relieved of eggs received a fresh lot during the following night. One of these, stripped the second time, died after taking on a third lot. When the eggs have been in the pouch thirty-six or forty-eight hours they become firmly fastened to it both at top and bottom, so that it becomes necessary to kill the fish and then cut away the flaps of skin before one can extract the eggs.

The fishes vary in size. The extremes in egg-bearing males of *S. floridæ* I have found to be 4.5 to 8.9 inches, and in females 3 to 8.4 inches. As a general rule, however, the females are somewhat the larger.

THE SEGMENTATION OF THE EGG OF THE PIPEFISH—SIPHOSTOMA FLORIDÆ.

I. THE OVARIAN EGG.

The ripe egg of this fish is of fairly good size, having a diameter of about 1 mm. It possesses a thin transparent membrane or shell, which, under the one-twelfth homogeneous oil immersion lens, shows no structure in sections, but in surface views presents, when stained lightly with hæmalum, a notably punctate appearance. These membranes were generally removed after killing the eggs, but, if left on the eggs, do not get very hard and offer no obstruction to embedding and sectioning processes. The eggs are formed in ovaries which, viewed from without, present the ordinary Y-shaped structure common to the Teleosts. These ovaries are two tubular organs situated in the posterior dorsal portion of the body cavity, and are confluent behind to form the short oviduct which opens on the posterior lip of the anal aperture.

However, when one of the ovaries is sectioned, a very interesting structure is revealed. Running lengthwise throughout the whole extent of the ovary is a raphe situated about two-thirds of the distance from one wall. From this eggs are budded off in succession to form a spiral of eggs which surrounds the raphe, the outermost egg being the oldest and largest. As this egg ripens it markedly increases in size and crowds the other eggs together with the raphe closely to one side of the tube. In the ovaries of older and larger fishes, two or three eggs may ripen side by side and then the raphe and its young eggs are very much crowded and contorted. As the eggs become ripe they enormously distend the ovaries both in diameter and length—in length until they frequently extend forward to the region of the stomach. At this time females ready to spawn are noticeable for their greatly distended abdomens.

The young eggs, as first pointed out by Cunningham (1897), have large nuclei with several nucleoli, but in the older ovarian eggs the

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germinal vesicle is not so apparent. The grown egg, still attached in the ovary, is surrounded by a layer of peripheral oil drops. This same structure persists in the eggs after extrusion, so that the germinal vesicle can not be seen. The sections I have made of eggs just extruded are so unsatisfactory and so little understood that further investigation is necessary before sections are figured. The older observers, Retzius (1833), Rathke (1836, 1837, 1840), Vogt and Pappenheim (1859), although they studied the ovary with the microscope, missed these peculiar structures. Later observers—Brook, McLeod, Cunningham (1897), and Huot (1902) have made sections but have not gone very far into the structure, nor will I myself do so now, since it is my intention to work up the organization and development of this organ later, the material for this being now on hand.

II. THE METHOD OF DEPOSITION.

This has already been described in the first part of this paper, but it may be well to emphasize the fact that the process is such as to prevent absolutely any contact of the eggs and sperms with the sea water.

III. FERTILIZATION.

The egg of *Siphostoma floridæ*, as before mentioned, possesses a very thin and perfectly transparent shell. This surrounds an egg made up of straw-colored yolk having many orange-red oil globules imbedded in its periphery and these surrounded in turn by a thin pellicle of protoplasm. The colored oil globules render the egg so opaque that I have never been able to find the micropyle. Yet, strange to say, the egg of a related European form, *Syngnathus ophidion*, was the first fish and possibly the first vertebrate egg in which this opening was discovered. Whether this egg is transparent or not I can not say, but in it Doyére (1849) found the micropyle just over the "*disque proligére*," and gave its diameter as $\frac{1}{115}$ mm.

A. Natural fertilization.—Different investigators vary in their conclusions, or, more correctly, their conjectures, as to the time of fertilization. *A priori*, one would expect the fertilization to be effected at the time of transfer. Probably the surest way to determine the time of impregnation would be to take a male immediately after the transfer, cut through the pouch just back of the forward end behind the genital opening, and then examine the eggs in the hinder part of the pouch for spermatozoa. This I had intended to do during each of the past summers. Although there were numerous transfers between fish kept in aquaria each summer, yet I saw the copulation on one night only (in 1903) between two pairs of fish. The seeming necessity for keeping these fish for the early stages of segmentation prevented my sacrificing either to determine this point.

Huot (1902), Lilljeborg (1891), Ryder (1882), and others think that the fertilization takes place at the time of copulation, while A. H.

Malm (1874) and Kroyer (1853) think that it follows later, and Ekstroem (1831) believes it takes place while the eggs are in the pouch. My own belief is that sperms and ova are emitted simultaneously, and while I have no direct evidence, the following facts corroboratory of this conclusion are adduced.

I believe that the extraordinary "*liebesspiel*," or period of sexual excitation of these fishes, described above, is intended to prepare them for the mutual discharge of the sexual products. In the description of the copulation and attendant phenomena, attention has been called to similar sexual excitements in an Amphibian, a Ganoid, and a Cephalopod, which are preparatory to the discharge of sperms as well as of eggs.

But the second set of phenomena is still more strongly corroboratory. On July 6, 1904, two fish were paired and during the night they copulated. They remained in the same aquarium for four days, and then the female was killed, her ovaries excised, cut up, and put into fixing fluids, while some of the ovarian eggs, which fell into the body cavity, were also killed. When these eggs were examined some months later, among them were found two embryos with the blastopore closed. None of the other eggs showed any trace whatever of impregnation. Again two lots of eggs, from a male killed in 1902, were examined two years later and found to be in the eight to sixteen-celled stage. In one lot, however, there was found an embryo with black eyes and free tail, and in the other two eggs in which the blastoderms covered one-half, the embryos one-fourth, of the circumference of the egg. These two lots of eggs had never been removed from the shells, and these shells were still bound together in masses as they came from the pouch. Thus all chance of the eggs having been mixed is eliminated. Again a lot of eggs put up in August, 1904, were found to be in the eight-celled stage, but among them were found two embryos with pectoral fins.

It is true that in opposite ends of the pouch eggs of different layings, and consequently different ages, are found, but never with differences of age more than thirty-six hours, against about three to five days in the above cases. From these facts I can draw but one conclusion—that at the time of coition both spermatozoa and ova are simultaneously extruded, and, as the female withdraws her oviduct from the buttonhole-shaped opening of the marsupium, sperms lodge on it and work their way through it into the ovary and there fertilize eggs. This happens only occasionally, but it seems to me a strong proof of my contention as to the time of fertilization. Gill (1905) quotes Nordquist, Ehrenbaum, and Eckstroem that internal impregnation occurs occasionally in non-viviparous fishes, such as the Sculpins. See Gill's interesting article on the Sculpin.

B. Artificial fertilization.—This was tried twice by the wet method and once by the dry. The eggs and the torn-up testes were thoroughly mixed in sea water, and after a few minutes were aerated in strained sea water. From a third lot of eggs the water was carefully drained, and over them was poured sperm from testes which had been torn up in a perfectly dry dish. These were allowed to stand for a few minutes, and were then placed in clean, running sea water. The females were certainly ripe for spawning, and the males were well grown and had not recently borne eggs, so they were presumably fertile. A control experiment was made by putting a batch of this last lot in running sea water without the addition of sperms. In *all* cases the results were the same. At the end of one and one-half hours protoplasm could be seen collecting at the upper pole. After two to three hours it was noticed that the eggs had flattened slightly at the animal pole and that there was being formed a pretty clearly defined round germinal disk, resting on a layer of orange-red oil drops. At the age of four to six hours the germinal disk was at its prime, but neither then nor at any subsequent time was there any trace of segmentation. From this time on the germinal disk gradually lost its sharp outlines, flattened down, and went to pieces. In one lot of eggs at the age of twenty-six hours the germinal disk had gone bad; in another after twenty-five hours it was no longer round, and its edges were irregular and fragmentary; in a third lot less than 10 per cent of the eggs were alive after twenty-three and one-half hours.

These eggs were all alike save that in one lot some, when taken from the ovary, showed a very faint aggregation of protoplasm at the germinal pole, while in another lot the eggs were of unequal size. This latter condition is, however, by no means an uncommon occurrence. Such differences are met with repeatedly in my preserved material, where eggs one-half to two-thirds the size of the normal ones are found. Save that the blastoderms are somewhat smaller, there is nothing unusual about the development of these small eggs. In this connection Brook (1887) says that the eggs of the herring vary in size in the same fish or in fishes of different localities, but thinks that this in no wise affects their development.

From my experiments it seems pretty clear that artificial fertilization is not possible in the pipefish, thus confirming the *a priori* opinion that this would not take place in fishes provided with such extraordinary apparatuses for the deposition and impregnation of the eggs, without their ever coming in contact with the water. Since the eggs will live for some twenty hours in sea water, it must be the spermatozoa which are disastrously affected by it. It has long been known that the sperms of both salt- and fresh-water fishes lose their vitality if left in the water any time and can not impregnate eggs. Quatrefages first ascertained this for the pike and other fresh-water fishes. Hoffmann (1881) says that the sperms of *Scorpæna* die quickly in salt-water. Reighard (1893) found that the sperms of the wall-eyed pike die after one minute in the water.

In this connection the experiments of Huot (1902) are very interesting. He took the eggs of *Syngnathus dumerilii* from the marsupium of the male, and, being careful not to break the egg membranes (these eggs were presumably fertilized), put them in clean aerated sea water. This he did also with eggs just before deposition (ovarian eggs), but in no case did development go on more than a few hours. Then he put into the water larvæ old enough to move freely, but these too died within forty-eight hours. I can confirm all his results. I have found that eggs in segmentation will go on dividing for a short while, but that within eighteen hours all die. The discoveries of Huot (1902) and of Cohn (1904), that the pouch and its contents act as a physiological placenta, offer the explanation for the above phenomena. The eggs and embryos, depending on this for oxygen and food, can not exist out of the pouch.

IV. MATURATION.

Unable to fertilize artificially the eggs of *Siphostoma floridæ*, and having found it impossible to get from the pouch eggs young enough to show the formation of polar bodies, I am unfortunately not in position to say anything of the process of maturation. For the latest and best work on this phenomenon the reader is referred to Behren's paper (1898).

V. FORMATION OF THE GERM DISK.

In the pipefish, fertilization is not necessary to bring about the formation of the germinal disk. Immersion in water supplies the stimulus as it does in many other fishes. All workers on the Salmonoids, Ziegler (1882), His (1899), and others, so report. Kowalewski (1886) found it true for the goldfish, as did Agassiz and Whitman (1885) for *Ctenolabrus*, though they state that for pelagic eggs the germ disk is generally not formed until after impregnation. Brook (1887) confirms this for the herring, but I have found that the eggs of the sargassum fish, *Pterophryne histrio*, form the germ disk shortly after extrusion. Hertwig says (Handbuch, p. 544): "One can emphatically say for almost all fish eggs that by their transfer into water such a powerful force is brought into play that the concentration of the germ disk results," but that "if they are impregnated first, a more rapid growth and larger size for the germ disk follows."

All writers, notably Brook (1887) and Ryder (1887), describe this formation as brought about by the streaming of the protoplasm to the germinal pole. There are three modes in which this may take place:

(1) By streams from the circumference only. This is the method in most fishes, especially those with pelagic eggs. (See Brook, Ryder, Kingsley and Conn, and many others.)

(2) By streams from the circumference with the help of little "processions" from the interior of the yolk (Ziegler, 1882, and Oellacher, 1872, for the trout).

(3) In all directions from the yolk, the streaming goes to the germinal disk (*Carassius*, Kowalewski, 1886).

As best I can determine, the pipefish comes under class two. This matter will be further referred to in the section dealing with the periblast.

Intimately connected with the foregoing is the collecting of the oil drops underneath the germ disk. In pelagic eggs, generally the oil is in one great globule near the center of the yolk, but in the pipefish many small orange-red globules are imbedded in the periphery of the yolk. When the protoplasm moves up to the animal pole, the oil globules go also and are collected under the germ disk to form the "*disque huileux*" of Lereboullet. This is a phenomenon very common among Teleösts. It has been reported by all workers on the Salmonoids, by Ransom (1867) for the stickleback, Kowalewski (1886) for *Carassius*, and by many others. Rathke (1837) first described these processes in pipefishes from the Black Sea. He says that the germinal disk is formed after the eggs come into water, and that the yellow-red "*fett*" drops which surround the yolk flow up to and spread out under the disk in a layer covering about one-third of its upper surface. Kupffer (1868), describing the egg of a European form, says, "This fat forms a mass of drops of different sizes, which incloses the germ disk underneath and laterally."

The two phenomena described above are intimately connected with and in fact bring about another known as the "clearing of the egg." As the protoplasm is withdrawn from the center and the oil globules from the periphery, the pipefish egg becomes "clear;" that is, the yolk, freed from these substances, becomes homogeneous and translucent. At this stage the egg of *Siphostoma* (Plate V, fig. 1) consists of a button-shaped protoplasmic disk resting on an orange-red layer of oil globules embedded in yolk and covering about one-fourth of the egg, the other three-fourths consisting of clear milky yolk. This "clearing" has been described, essentially as above, by Fusari (1890), Kowalewski (1886), and Agassiz and Whitman (1885), for *Cristiceps*, *Carassius*, and *Ctenolabrus*, respectively.

In connection with the above processes, many workers, especially the students of the Salmonoids, have described amœboid movements of the germ disk, and His, in a recent paper (1899), has described such activities in the blastomeres up to the sixteen-celled stage. Ransom (1867) has also figured and described amœboid movements in the yolk of *Gasterosteus*. These movements seem to assist in freeing the yolk of protoplasm and the germinal disk of yolk. The opacity of the egg, which prevented my making out much about the "streaming," operated here against the detection of such movements. Once or twice, however, I thought that I did make them out, and in several hardened germs there were found such protuberances as are figured by Henneguy (1888) in trout germs hardened in chromic acid.

The oil drops in the pipefish egg are not numerous enough to make it float, but from their location they maintain the germ in an upright position. If the eggs are overturned, this buoyancy causes them to rotate quickly in the liquid filling the "breathing chamber" of Ransom. How long this rotation persists I can not say, but certainly until after the closure of the blastopore. Rathke (1837) first noted this in the eggs of Black Sea forms. He also described, as best I can make it out, an albuminous material coagulable in water or in air, which fills the "*zwischenraum*" referred to above. Whatever may be the liquid filling this space in *S. floridæ*, it does not coagulate in water, air, or in any of the fixing fluids I have used. It might be well to add here that this rotation of the egg is not a new phenomenon, having been reported, notably by Ziegler (1882) and His (1899) for the salmon family.

My earliest preservations of eggs with forming germ disk were made four to five hours after the eggs had been placed in the water, hence I am not able to describe by sections its formation. In any case, however, I could not hope to add anything to the classic paper of Agassiz and Whitman on *Ctenolabrus*, or to the more recent memoir of Behrens on the brook trout. Since I preserved eggs at intervals of from five to twenty-five hours, I have sections which illustrate the progressive degeneration of the blastodisc. So far as I know this has never been shown, and hence it may be of interest to give a few figures illustrating this phenomenon.

Fig. 1, Plate V, represents the sharply marked off blastodisc resting on the yolk sphere. It shows the relative diameters of blastodisc, "*disque huileux*," yolk sphere and egg membrane. Fig. 28, Plate VII, is a central section of a germ disk five hours old. The concentration of protoplasm is not yet perfect. As best I can make it out, all has not yet emerged from the central yolk. The dotted line marks off a region where protoplasm and yolk are so closely intermingled as to be indistinguishable. Oellacher (1872, fig. 17) figures and describes a similar germ disk for the trout. Fig. 29, Plate VII, shows a degenerating blastodisc ten hours and twenty minutes old. Such structures are not unfrequent in unfertilized eggs found among others in the four to sixteen celled stages in ages from eight to twelve hours. They are also found in eggs which have been in water about ten hours, and, I am inclined to think, are of fairly regular occurrence in degenerating blastodiscs of unfertilized eggs.

Stricker, in 1865, described what he called an entirely new mode of cell formation in the blastoderm of the brook trout—that is a budding off of cells—which he thought originated in the amœboid activities of the protoplasm. His figures show blastoderms with from one to twenty-three "buds," lumps, or vesicular swellings on the outer surface, and his one section is very inconclusive. Unfortunately, I have no surface views of pipefish eggs showing any of these structures. The following year Ransom reported a similar bud formation in the unimpregnated eggs of the pike. These "showed a lobulation of the concentrated formative yolk, a sort of irregular asymmetrical cleavage." After twenty-five hours "portions of the discus proligerus were pinched off and appeared as projecting buds." His reported in 1899 that unfertilized salmon and trout eggs after lying in water four weeks formed hillocks on the surface of the germinal disk by the outpushing of fluid drops under the surface membrane. Neither he nor Ransom give figures. Fig. 29, Plate VII, makes clear these various observations.

As to the further fate of the blastodisc in the unimpregnated egg of the pipefish, I can only say that it flattens out and finally disappears. Fig. 30, Plate VII, is a central section through a blastodisc twenty-six and one-half hours old, which shows this flattening. Fig. 31 on the same plate shows a blastodisc taken from a lot of eggs in the invagination stage (forty to forty-eight hours). It is much larger and its lower surface is comparatively free from yolk. The contrast is evidently due to the fact that one egg has been lying free in the sea water, while the other has been under more favorable conditions in the marsupium. Just here it may be of interest to note that while unimpregnated eggs are often met with in the pouch with embryos of all stages, none of them ever "go bad." Ransom (1867) reports that he has kept unfertilized trout eggs alive in running water forty-three days. More recently, His (1899) gives four weeks for the maximum time, and describes the mass of germ-plasm in the unfertilized eggs of the trout and salmon as decreasing day by day and becoming more and more set through with oil drops and yolk spheres. The degenerating blastodiscs of the pipefish in some cases show these inclusions, but in general are quite free from them.

VI. SEGMENTATION.

Before going into a description and discussion of the segmentation of the egg of *Siphostoma floridæ*, I wish to say that this is extraordinarily irregular. These irregularities begin as early as the two-celled stage and become very marked when eight cells are formed. The egg under consideration equals and perhaps exceeds that of the Salmon family in abnormality of cell division. The surface views were nearly all drawn from the hardened germs in 80 per cent alcohol or xylol, the opaque egg making it impossible to draw *in situ* blastoderms beyond the eight-celled stage. The drawings were all made with a Bausch and Lomb microscope (the tube drawn out to 160 mm) and camera lucida. The surface views were all made with the 1-inch eyepiece and the two-thirds objective. Sections were drawn with the 2-inch eyepiece and the one-sixth objective. Plates V and VI have been reduced one-half, the others two-thirds.

ONE-CELLED STAGE.

This is shown in fig. 1, Plate V, from above, and in fig. 32, Plate VII, in section. It is high arched and falls steeply into the outer periblast, from which it is clearly marked off by the circumferential furrow of the authors. This furrow is sometimes so pronounced in the germ disk of the Salmon family that the disk literally overhangs its base. See His (1898, fig. 1) for the trout and (fig. 2) for the salmon. Kupffer (1868), however, says that in a European *Syngnathus* (species not given) the germ disk is not sharply marked off from the periblast, and that this condition holds till the end of the four-celled stage. Most workers on the Salmonoids, Behrens (1898), and, notably, His (1899), represent the unsegmented blastodisc as somewhat sunken in a saucer-shaped depression. In the pipefish, however, the blastodisc, fig. 1, Plate V, underlaid with oil globules, rests on a slightly flattened area at the upper pole. Below it is not sharply marked off from the yolk, but across its base extends a band, about as wide as the periblast to the right, composed of mixed yolk and protoplasm. The section shows several vacuoles to the right, which in the living egg were probably filled with oil. Brook (1887) describes in the herring a blastodisc with yolky base; His (1899), the like in the salmon.

This blastodisc was found in a batch of eggs in the eight to sixteen-celled stage (eight to twelve hours). His (1899) says the germ disk in the Salmon is formed in from one to four days. Hertwig (1903) says that the formation of the germinal disk in the herring takes place in two hours, and in the trout from seven to eight hours. Evidently the time varies with the kind of fish, the temperature, and the purity of the water. In the pipefish I have found it to take place in from four to six hours. It is noteworthy that in none of the blastodiscs which were sectioned have I ever found a nucleus. Brook (1887) could find no nuclei in the herring until after the appearance of the third furrow.

TWO-CELLED STAGE.

As in Teleosts generally, the blastodisc elongates slightly before the appearance of the first furrow, and, as a result, one axis is somewhat longer than the other. This is shown in fig. 2, Plate V, the normal two-celled stage, in which the blastomeres are equal. In fig. 3, however, we have an irregular segmentation, with one cell much larger than the other and with a vacuole in the line of division. Of this type quite a number were found.

Fig. 33, Plate VII, shows a flat two-celled blastoderm, not definitely marked off on the right from the outer periblast, in which the nuclei have divided, the external furrow has formed, but the cell wall has not yet come into existence. In the line of division, the protoplasmic reticulum has formed a very delicate network of dendritic fibrils arranged transversely to the plane of cleavage. Oellacher (1872, fig. 20) describes and figures a section through two cells of a four-celled stage in the brook trout very like this. He says an indistinct streak made up of faint granulations runs vertically from the external groove toward the base. Henneguy (1888, fig. 60) gives a figure of a two-celled stage very like fig. 33, Plate VII, and says that the fine line dividing the two cells is bordered on each side by clear protoplasm which is traversed by very fine lines parallel to each other and perpendicular to the median line, and that these fine lines lose themselves in the surrounding protoplasm. His (1898, figs. 7, 8) illustrates and describes similar structures in the syncytium at the base of the trout germ in early stages. In fig. 34 we have a high arched two-celled stage in which the perfectly distinct cell wall is interrupted by a vacuole near its center. This is plainly a derivative of fig. 32, as the preceding is of fig. 28.

Fig. 35 is a section through fig. 3, Plate V, in the plane *a–b*, and shows the split between the two cells dilated into a large vesicle at the bottom. Very frequently the division between the two cells takes the form of a deep cleft with nearly vertical walls, and at the bottom the cleft may or may not dilate to form a small vesicle. These structures are shown in fig. 36, and are oftentimes much larger than figured here. In fig. 37 we see the split being formed by the breaking down of the walls of a series of vesicles placed vertically over one another in the center of the blastoderm. This formation of vesicles in the line of cleavage was, so far as I know, first figured and described, for the trout, by Oellacher in 1872. Balfour (1878, figs. 6, 6a, and 6b, Plate I) illustrates and at some length describes vacuoles in the early furrows of the skate. He describes such a beaded structure, as shown in my fig. 37, and thinks that these vacuoles are more common than supposed, and that they play a considerable part in the segmentation. Brook (1887) describes the like in the herring but gives no figures. Kowalewski (1886, fig. 1, Plate XVII) finds vesicles at the bottom of the furrows in the early stages of the goldfish. Agassiz and Whitman (1889) figure, in surface views of blastoderms of *Ctenolabrus*, rows of small vacuoles extending along the whole length of the cleavage planes in the two- and four-celled stages, but do not refer to them in their text. Fusari (1890, figs. 4 and 5, Plate III) shows in both surface views and sections blastoderms with vacuoles. Some of the sections show vacuoles with large dilatations at the bottom like those in figs. 35 and 36, Plate VII.

In the pipefish, the first furrow does not cut through to the yolk. (See figs. 34, 35, 36, and 37.) In this respect it agrees with *Cristiceps* (Fusari, 1890), the Herring (Brook, 1887), *Carassius* (Kowalewski, 1886), the Bass (Wilson, 1891), the Salmon and Trout (His, 1898), but is unlike *Merlucius* (Kingsley and Conn, 1882), *Gadus* (Cunningham, 1887), and others, which do cut all the way through. Agassiz and Whitman (1889) show that in *Ctenolabrus* the first furrow may or may not penetrate to the yolk. There is never any such under furrow as the bass and *Ctenolabrus* show in the first division.

The eggs are laid at night, as early as 10 o'clock, and probably at any hour thereafter. At any rate, by 7 o'clock the next morning, they are to be found in stages of from two to sixteen cells. Probably from four to six hours elapse before they begin to segment, since it takes this long for the germ disk to form on eggs in water, in comparison with six and one-fourth hours for the herring (Brook, 1887) and twelve to thirteen for the salmon (Hoffmann, 1888).

FOUR-CELLED STAGE.

In fig. 4, Plate V, is shown a normal four-celled blastoderm. The second furrow is horizontal and crosses the first approximately at right angles. Thus there is formed a four-celled symmetrical blastoderm. Sections of this would in no wise differ from those for two-celled stages, save in the plane *a–b*, where the beginnings of the segmentation cavity and the central periblast would be found. Such a section is not at hand, unfortunately.

Fig. 5, Plate V, a more common form, shows slight inequalities in the size of its blastomeres. Such irregularities become more pronounced until they result in reniform blastoderms, as fig. 6, Plate V. Fig. 38, Plate VII, is a nearly horizontal section through the base of such a form as fig. 4, Plate V. The wide separation of two of the cells is an artefact. Of special interest are the segmentation cavity in the center and the remnants of protoplasmic bridges which connected the blastomeres.

EIGHT-CELLED STAGE.

Into the blastoderms of the pipefish egg of this stage, many very great and seemingly irreconcilable irregularities enter and greatly confuse the investigator. These were first noted on living eggs with four and eight cells below, two, three, and four above. Hardened eggs showed the same irregularities. Surface views of a great many of these eight- to sixteen-celled blastoderms were drawn. When a comparison of these drawings was made, they were found to conform to four general types. This was confirmed by an examination of all the eggs of this stage which had been preserved. At the close of this section, there is appended a table showing the relative numbers of these various types.

In fig. 7, Plate V, is shown the normal type of 8-celled teleost blastoderm. It is formed by two furrows nearly parallel to the first and perpendicular to the second plane of segmentation, dividing such a form as fig. 4, Plate V, into eight blastomeres. In this blastoderm, and in nearly all others of this and the next stage, a considerable elongation is noticeable.

Figs. 8 and 9, Plate V, show variations of this normal type, which are more common than the type itself, but are easily referable to it. Fig. 39, Plate VII, shows a section of fig. 7, Plate V, in the plane *a–b*. In it one of the two central cells is completely cut out of the protoplasm, while at the inner end of the cell wall, partly cutting out the other cell, there is a little split, which in sections nearer the center will push a short distance to the left, but on the right will extend *clear* across, completely cutting out the cell and extending the segmentation cavity (*s. c.*). The layer of protoplasm with yolk marked *c. p.* is the central periblast, and the cavity above it is the segmentation cavity. This, however, is not the first appearance of either, since a section in the plane *a–b*, in fig. 4, Plate V, would show both. I regret that I have not been able to find such a section. The outer periblast never shows the periblastic ridge figured by Wilson (1891) for *Serranus*. Fig. 40, Plate VII, is through the plane *a–b* of fig. 16, Plate VI, a normal sixteen-celled stage, but it will show the state of things in the plane *c–d* through fig. 7, Plate V. In this part of the normal blastoderm of this stage, the central cells are separated from the periblast by a large segmentation cavity, which extends for a short distance under the peripheral cells, in this case the end cells of fig. 7, Plate V.

Fig. 41, Plate VII, is a section at right angles to the long axis of a blastoderm, similar to fig. 7, Plate V. Here the two cells are separated from each other by a wide segmentation cavity (*s. c.*) roofed over by a protoplasmic bridge (*p. b.*) connecting the two blastomeres. A thin split extends for some distance under each cell and partially separates it from the central periblast (*c. p.*), which is heavily laden with yolk in its lower parts. Such protoplasmic bridges as the one shown here are not uncommon in this and the next stage. All that can be said of their origin is that they have been left behind when the cells were cut out of the protoplasm. Structures similar to this would be found by making sections at right angles to the long axes of figs. 8 and 9, Plate V. So far as I know, these protoplasmic bridges have not been figured and described before.

The periblast never comes away freely from the yolk, but is so obscured with fragments of this latter that it has in all cases been drawn semi-diagrammatically, the general course of the break only being followed.

Fig. 10, Plate V, shows a type of eight-celled blastoderm far more common in the pipefish than the preceding. In this the plane of the third furrow shifts until it becomes equatorial and cuts off four somewhat smaller blastomeres from four underlying larger ones. Henneguy (1888, fig. 39) shows a blastoderm for the trout which is almost an exact counterpart of this. A section through this blastoderm in the plane *a–b* reveals the structure shown in fig. 42, Plate VII. Here the two central cells stand above the basal ones, with the line of demarkation on the right especially sharp. The segmentation cavity (*s. c.*) and the central periblast (*c. p.*) are both very much reduced.

Another very common form of eight-celled blastoderm is shown in fig. 11, Plate V. Here there are six cells below and two above. This is evidently a derivative of a six-celled stage frequently met with, in which two of the blastomeres of fig. 4, Plate V, divide by vertical furrows, the other two cells undergoing no change. Later, however, a division of these in a horizontal plane would give the structure shown in fig. 11. Variations of this type are frequently due to the shifting of this pair of upper cells. Such a divergence is shown in fig. 12, Plate V, where these two cells reduced in size are shifted to one end of the longer axis of the blastoderm. Sometimes these two cells are placed parallel to the main axis, but over one of the central lateral cells. Again they may be shifted to lie at right angles to the long axis, over one of the furrows separating two lateral cells, so that one cell is at the edge of the blastoderm. In order not to multiply figures there is given only one drawing of sections from such blastoderms. Fig. 43, Plate VIII, is a section through such a structure as fig. 12, Plate V, in the plane *a–b*. Here one central cell is very much higher than any of the other cells. The other central cell is completely cut out of the protoplasm and is roofed over by a protoplasmic bridge extending from the high cell to the left outer cell. Following the sections to one side of this, the bridge and the cell under it are found to unite. They would thus seem to have been split apart from the same mass of protoplasm.

Another eight-celled blastoderm, quite as common as either of the foregoing, is represented in fig. 13, Plate VI. Here one cell has, by an equatorial furrow, become cut out to lie slightly above the rest. The right side of the structure is normal, save that the third cell is slightly flattened at its inner edge by contact with this central cell. As in the preceding case, so here there may be variations in the position of this high level cell. It may lie in the center, at the edge, or at any intermediate position on the blastoderm. A section through the long axis of fig. 13 would give a structure essentially like that shown in fig. 43, Plate VIII, omitting the protoplasmic bridge. Klein (1872, figs. 5 and 6, Plate XVI) shows essentially the same structures in the same stage of the trout germ, as does Henneguy (1888) in his fig. 38, Plate XVII.

Fig. 14, Plate VI, is a seven-celled form, in which an unmistakable equatorial furrow has cut off three upper from four lower cells, of which three are very large. A view of this blastoderm from below is shown in the next figure (fig. 15). Here the two meridional furrows show quite clearly, but there is no trace of the third or equatorial furrow. The segmentation cavity (*s. c.*) is so small as to be almost negligible. Unfortunately, no section of this figure can be given, but a comparison between it and fig. 42, Plate VII, will make clear its internal make-up.

These nine figures of the eight-celled stage have been introduced to show (1), the great irregularities which enter into the segmentation of the pipefish egg at this stage; (2), that these all result from the position of the third furrow, which, ordinarily meridional and parallel to the first and perpendicular to the second plane of division, here becomes equatorial, and (3), that the irregularities thus resulting may be reduced to four types, which may be traced to the very close of segmentation. In order to establish definitely these points, a table is given showing the relative numbers of the different kinds of eight-celled blastomeres which have been counted.

From these eight-celled blastomeres are derived four types of segmentation which persist to the close of segmentation. From figs. 7, 8, and 9 come two types of flat structures; from figs. 10, 11, 13 (with the eighth cell in center) there comes a high-arched type of blastoderm, and from figs. 12 and 13 (with the eighth cell at one end) a type of blastoderm thick at one end and tapering toward the other. These structures will be more clearly shown in the next section.

Table showing relative numbers of blastoderms for each type of the eight-celled stage of the Pipefish egg.

[Types referable to figures on Plates V and VI.]

Killed in—	Lot.	VII, VIII, IX.	X.	XI.	XIII.	XIV.
Perenyi	1	0	2	5	2	6
Formalin	2	4	5	6	4	8
Perenyi	3	0	3	1	4	1
Formalin	4	0	7	3	3	0
Do	5	[a]2	8	3	3	0
Sub-acetic	6	0	0	2	4	0
Perenyi	7	2	1	0	2	0
Total	7	8	26	20	22	15

[a] Six-celled.

SIXTEEN-CELLED STAGE.

Intermediate between the eight and sixteen-celled stages are found many blastoderms with twelve, fourteen, and fifteen cells. These are in fact more abundant than blastoderms with exactly sixteen cells.

Figs. 16 and 17, Plate VI, show the two most regular sixteen-celled stages that have been found, yet they do not have the regular structure of the corresponding stages shown for *Serranus* by Wilson (1891) and for *Cristiceps* by Fusari (1890). These blastoderms have been formed by each of the cells in figs. 7, 8, or 9, Plate V, dividing into two. In fig. 16 all the cells save one are practically on the same level, or at most with a gentle curve across the upper surface. In fig. 17, the blastomeres are arranged more irregularly. Fig. 40, Plate VII, is a section in the plane *a—b* of a blastoderm like fig. 16, Plate VI, preparing to divide into thirty-two cells. The two central cells will divide to form two surface and two interior cells, while the outer cells will each divide into two cells, both on the surface. This is shown by the position of the centrosomes. The cells form a gentle arch roofing over a considerable segmentation cavity. The planes of segmentation are dilated at their outer ends into vesicles which are covered by thin protoplasmic sheets or bridges. Fig. 44, Plate VIII, is a section of some such structure as fig. 17, Plate VI, in the plane *a—b*. Some blastoderms of this stage have been found in which the four or five cells were not cut off from the basal periblast, but these are too infrequent and too little understood to be reproduced here. Fusari (1890) has figured a section like this for *Cristiceps*, a goby.

In fig. 45, Plate VIII, there is shown a section of a flat-topped abrupt-edged sixteen-celled blastoderm of a type which persists till the preparation for invagination begins. What the appearance of such a blastoderm in surface view would be I can not say; probably it would in no wise differ from fig. 16, Plate VI. The essential difference between figs. 44 and 45, Plate VIII, is the circular groove sharply marking off the outer periblast (*o. p.*) in the latter. Possibly these figures are derivatives of the one-celled stages shown in figs. 28 and 32, Plate VII. In fig. 45, Plate VIII, there is a large segmentation cavity and a yolk-laden periblast. The dotted lines show where the outer periblast has been torn away. Note the large dilatation at the outer end of the right furrow and the protoplasmic bridge covering it. Fig. 18, Plate VI, is a derivative of some such forms as figs. 10, 11, 13, and 14. It is arched, but the crest of the arch is not in the center but to one side, and the cells lie in two if not three levels. A section through an almost identical form (in the plane *a—b*) is shown in fig. 46, Plate VIII, and makes clear its sloping outline and its two excentrically placed high cells. It has one interior cell, which in the next section is clear of the central periblast (*c. p.*), and has probably originated by the horizontal division of an outer cell.

Fig. 19, Plate VI, shows a modification of the arched type. Its sixteen cells are in two layers and the seven upper ones are on an approximate level. Fig. 47, Plate VIII, is a section through some such blastoderm as the above. Its surface slopes gently and the left peripheral cell projects over the outer periblast (*o. p.*). This latter phenomenon will be found frequently in later stages. Vacuoles are found in two of the division walls.

The high-arched type of sixteen-celled blastoderm is shown in fig. 20, Plate VI. This is probably a descendant of a blastoderm like fig. 14, on the same plate. No description of it is needed, beyond calling attention to the fact that the five upper cells are cut out by an equatorial furrow. This is seen by referring to fig. 21, which is a ventral view of the same blastoderm. Here only five of the vertical planes seen from above cut all the way through. The ones marked *o* in fig. 20 have not reached the base. The small segmentation cavity (*s. c.*) recalls that of fig. 15. Let us compare with this the next, fig. 22, which is a view from below of a similar high-piled sixteen-celled stage. Here there are nine basal cells resting on the yolk, six in the second tier, and a central one forming the keystone of the arch, the whole inclosing a spacious segmentation cavity. Barring the fact that the segmentation cavity (*s. c.*) extends under the marginal cells, fig. 48, Plate VIII, may be given as a section through fig. 22 in any vertical plane passing through the keystone cell. The central cell has not yet completely cut itself off from its neighbor to the right, and the cell to the left has a resting nucleus curiously elongated.

There have now been figured and described in surface views and sections, such sixteen-celled structures as may be considered typical for the pipefish. Of these, two are sufficiently like the usual teleost form as to be called normal, but a great majority, fully 90 per cent of those studied, are like figs. 18, 19, and 20, Plate VI. In this connection Hertwig's statement (Handbuch, pp. 645–646), with reference to the fourth segmentation and formation of the sixteen-celled stage, is of interest. He says: "The end result is everywhere the same, a 'checkerboard-like' arrangement of sixteen blastomeres, four in the center, and a circle of twelve marginal cells." How untrue this is for the pipefish, a glance at the figures given and at the table shown on page 478, will demonstrate.

EQUATORIAL PLANE OF SEGMENTATION.

All investigators are agreed as to the homology between the first and second furrows in teleost and amphibian eggs, but whether or not the third furrows correspond is a very debated question.

Hoffmann (1881) figures and describes in pelagic fish eggs the first segmentation as equatorial, dividing germ from periblast; but later (1888), he acknowledges his error and declares that in *Salmo* the third furrow is equatorial. Ziegler says that the third furrow in the salmon and trout is equatorial and divides eight upper from eight lower cells, the latter not being as yet marked off from a periblast. Rauber (1883) made a careful study of the subject based on the well-known fact that the fourth amphibian furrow in a great many cases is not truly meridional but avoids the pole and forms many structures like figs. 7 and 9, Plate V. He concludes that the first *equatorial* furrow of the frog has been lost in the Teleost, and homologizes the third teleostean with the fourth (pole-avoiding, meridional) furrow of the frog. For this interpretation of Rauber see Wilson (1891, pp. 214–215).

Agassiz and Whitman (1885) think that the amphibian equatorial furrow has become vertical in the Teleost, and that the horizontal division of the four central cells of the sixteen-celled stage into four outer and four inner lying cells is the first equatorial segmentation. With this latter statement Kopsch (1901), from his work on *Belone*, is in full accord. Brook (1887) describes, from sections of herring eggs (Plate XIII, fig. 9), an equatorial segmentation separating the four blastomeres from the periblast. List (1887, Plate XXXI, figs. 4 and 5) finds the second furrow in *Crenilabrus* to be equatorial, and says that Kupffer found the same in the herring. In *Cristiceps*, Fusari (1890, figs. 4 and 5, Plates I and III) finds that in the sixteen-celled stage, all the cells are united at the base, but the next division sets sixteen central cells free from the yolk and from sixteen peripheral cells. This he calls the equatorial division. Wilson (1891, p. 215) agrees with Rauber (see above). Samassa (1896), in the segmentation of Salmonoids, finds as a rule that an equatorial division follows the eight-celled stage, although it sometimes comes earlier.

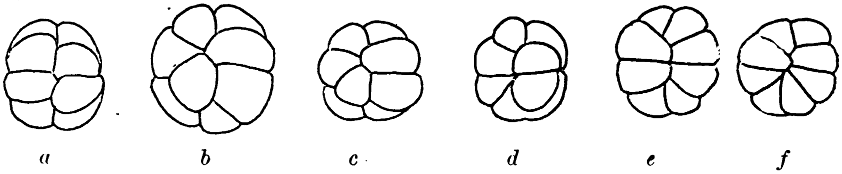

FIG. 2.—EGGS OF THE TRITONS IN THE EIGHT-CELLED STAGE. (AFTER GRÖNROSS.)

An equatorial segmentation has been pointed out in certain eight-celled blastoderms of *Siphostoma*, and this gives them a very decided resemblance to the upper surface of dividing amphibian eggs. Grönross (1890) (see Hertwig's Handbuch) gives a series of figures for the eggs of Tritons to which the figures above noted show very striking resemblances. The Tritons have eggs with relatively large amounts of yolk and in them the segmentation approaches the meroblastic condition. The text figure reproduces some of the more striking forms to which reference will be made. The resemblance is so striking that no extended comparison is called for. With Grönross's fig. *a*, compare fig. 10, Plate V, and also Henneguy's (1888) fig. 39. They are almost identical. For a figure which almost duplicates his figs. *b* and *c*, see fig. 13 on the next plate. Among drawings not included in the plates is one almost identical with his fig. *d*. Again, figs. *e* and *f* are very similar to fig. 9. The comparison might be extended further, but this is sufficient to show the very striking similarity between these two forms. That we have here an analogous segmentation is beyond question. The segmentation in the pipefish egg in the blastoderms in question is equatorial or at least approaches very close thereto, and it seems hardly going too far to say that such pipefish blastoderms as figs. 10, 11, 14, 18, 20, there is a reversion to a type of segmentation essentially like that of Amphibia.

THIRTY-TWO-CELLED STAGE.

Normal types of this stage, as shown in fig. 23, Plate VI, were found to make up about 30 per cent of one lot of eggs, and were noted sparingly in all others. Fig. 23 is plainly a derivative of forms like figs. 16 and 17, and, while it may be called normal, is noticeably different from Wilson's figures of the same stage for *Serranus* (figs. 6, 8, 9, and 10). No section of this stage will be given. Its internal structure will be made clear by reference to fig. 40, Plate VII, a sixteen-celled blastoderm ready to divide into thirty-two. The two central cells will divide horizontally, the two lateral ones by an oblique plane resulting in six surface and two interior cells. (Compare Wilson's fig. 18.)

Fig. 49, Plate VIII, is a section from a flat-topped abrupt-edged blastoderm, drawn with the same magnification as the others. It serves to show the inequalities in the size of the blastoderms. The peripheral cells are very much flattened above, though retaining their rounded forms below. To the right the section cuts the point of a sixth cell. The segmentation cavity (*s. c.*) is partially filled with cells. The larger and lower cell seems to have been cut off from the central periblast (*c. p.*), from which it is separated by a cell wall so delicate that the oil immersion only will detect it. It is like the periblast in that its periphery contains many yolk granules.

Fig. 24, Plate VI, is an arched type, with the highest point rather nearer the lower side. The twenty-seven outer cells are in three tiers, and while the second is pretty sharply marked off from the first there is but little difference in level between it and the third tier. There is here noticeable a symmetry comparable to that in figs. 7 and 8. The plane 1—1 in all probability represents the first, 2—2 the second line of division referable to fig. 4.

A central section through fig. 24 in the plane *a—b* is shown in fig. 50, Plate VIII. The peripheral cells form an arch with the highest point slightly to one side, and inclose a segmentation cavity which is almost filled with cells. The two smaller cells have been cut off from the peripheral ones, the larger probably from one of its fellows. The periblast is thick and yolky. A more pronounced large-ended type is fig. 51. Here the segmentation cavity is somewhat eccentric, and, as in the preceding, the thick end overhangs the base. The spacious segmentation cavity (*s. c.*) contains one cell which abuts on a curious tongue of protoplasm from a partially segmented region on the left.

Fig. 25, Plate VI, is a typical high-piled blastoderm, whose cells are arranged in three layers. Its highest cell is slightly eccentrically placed, and one of the axes of the blastoderm is somewhat longer than the other. Fig. 52, Plate VIII, is a central section through a similar but slightly older blastoderm. The marginal cells are sharply marked off from the outer periblast (*o. p.*). The arch is high and round. On the left, two cells are imperfectly separated, and a tongue of protoplasm, from which a cell has been cut off, projects into the large segmentation cavity. The periblast, torn off at the right, is in the center reduced to a mere film of protoplasm with much yolk adherent below, thus giving it the breadth as drawn.

Fig. 53 shows a structure by no means uncommon in the egg of *Siphostoma*. It is a thirty-two-celled stage in which no periblast has yet been formed. The cells are in two layers, the long cell on the upper right is nearly ready to divide, and underneath the whole is a thick layer of protoplasm in which three vertical cell walls extend downward and are lost. Later transverse walls will appear and cut these cells out of the syncytium, finally leaving a periblast layer below. There is a very small segmentation cavity (*s. c.*) and the large cell to the right has a vacuole (*v.*). Ziegler (1882, fig. 2) figures an almost identical structure for the salmon. Kowalewski (1886, figs. 1 and 2) portrays essentially the same conditions in the goldfish. Hoffmann (1888, figs. 6 and 9, especially) describes a similar structure in the salmon germ. And latest of all His (1898, figs. 7 and 10) confirms the figures and descriptions of the earlier workers on the Salmonoids.

Fig. 26, Plate VI, is a very interesting divided blastoderm of this stage with eighteen cells in one division and fourteen in the other. Such structures have been met with occasionally in stages of from sixteen to sixty-four cells, but especially abound in the eggs from one fish. Out of twenty of these eggs killed in picro-acetic, five were like the one figured. That these were not artefacts is shown by the fact that eggs of the same lot killed in formalin also contained divided blastoderms, the numbers of which were unfortunately not noted. In each division a segmentation cavity exists, and the line of separation is broad and definite down to the periblast. These points are brought out very definitely in fig. 54, Plate VIII, a section through a similar but older blastoderm. In the left half there is a small segmentation cavity (*s. c.*); on the right, however, there is none. There is no periblast. Cells have been cut out of the mass of protoplasm, leaving a thick germ basis in which are found vertical cell walls and a number of vacuoles (*v.*), and which is filled below with fragments of yolk. Fig. 55, Plate IX, is a divided sixty-four-celled stage of the thick-ended type. The furrow between the two parts is here not so wide. In other blastoderms this may swell out to a vesicle at the bottom or be reduced to a mere line, as in the two-celled stages above. There is a segmentation cavity in each portion, but there is no distinct periblast, the basal layer of protoplasm being thick with a large vacuole and full of yolk in its lower part. In some cases, where the plane of separation is reduced to a line, the cells are drawn out into long points toward the base as if a fine thread, used to separate the parts, had elongated the cells downward.

The only reference to such peculiar conditions as shown in these figures is found in a short section on Coregonus in Eycleshymer's paper on Amblystoma (1895, fig. 35 and others). This writer thinks, however, that these divided blastoderms do not result in double embryos. The same seems to hold true for the pipefishes of Beaufort, for although thousands of eggs and larvæ and hundreds of adults, alive or preserved, have been examined, only two apparent cases of deformation have been found by the writer. The literature of these fishes contains but few references to abnormalities. M. Malm (1862) describes a *Syngnathus* with two caudals. Ryder (1884) reports a *Syngnathus* with two anals. However, Rathke (1837) reports in the Syngnathidæ of the Black Sea many abnormalities of the snout, eyes, and tail, due, he thinks, to retardation of development.

A fair example of the late stages of segmentation is shown in fig. 27, Plate VI. Here the thirty-eight cells are in three tiers, with one cell high above all. There is an elongation in one axis, possibly a derivation of the condition found in the eight-celled stage, and a curiously regular arrangement of certain cells. On the whole, however, the segmentation is very irregular, and it becomes more so later; finally all trace of symmetry is lost, and the blastoderms become almost circular in outline. No surface views of later stages will be given, since, as the cells grow smaller, the blastoderms approach more and more the ordinary teleostean form.

STAGE OF SIXTY-FOUR CELLS.

Artificial fertilization being impossible in *Siphostoma*, one can not divide late material into stages by hours, and the greatly varying shapes of the blastoderms make it impracticable to classify sections by the number of rows of cells in each, as some writers do, so it becomes necessary to devise an arbitrary scheme. This scheme is to count the peripheral cells in the central section of a blastoderm, then, assuming a like number in a section at right angles to this, by squaring this number the approximate number of surface cells is found. The size of the cells serves as a check to this.

Fig. 56, Plate IX, with eight peripheral cells, is from a normal type of the sixty-four celled stage. The central periblast (*c. p.*) is thick and yolky, and at the right is a cell not yet cut off from it. The segmentation cavity (*s. c.*) is filled with cells, some of which are ready to divide.

Fig. 57 is derived from a flat blastoderm of the preceding stage, and, by comparison with figs. 45 and 47, Plate VIII, is seen to have undergone considerable division in horizontal planes, as is shown by the number of cells filling the segmentation cavity. The large nuclei are in the spireme stage, and in the left marginal cell there are two large vacuoles.

The high-arched type of this stage is shown in fig. 58, a derivative of a structure like fig. 53, Plate VIII. The surface falls steeply into the outer periblast (*o. p.*), the cells are all rounded and have small nuclei. Very interesting are the two cells which are incompletely cut off from the central periblast (*c. p.*). Scattered yolk granules are found in some of the cells. The mitotic figures indicate that division into the next stage has begun.

In fig. 59 we have an example of the thick-ended type. The section is slightly to one side of the center, and shows one cell just free and another not yet cut out from the thick yolky periblast. Note the vacuoles which help to delimit cells. In the central section the small segmentation cavity (*s. c.*) becomes somewhat larger. The outer cells are flattened on the exterior, and the whole structure is very like fig. 55.

STAGE OF ONE-HUNDRED-TWENTY-EIGHT SURFACE CELLS.

The normal gently arched type is represented in fig. 60, a nearly central section of a blastoderm of this stage. The central periblast (*c. p.*) is here thick and fairly well delimited from the yolk below. Of especial interest are the cells in the act of being cut out of it into the segmentation cavity. Very notable is the agency of vacuoles (*v.*) in this process. The cell next to the right marginal cell has in its lower part a nucleus, the first met with in the periblast region.

Fig. 61 is an example of the flat-arched type. The central peripheral cells, like those of the preceding stage, have undergone more division than their fellows. The periblast at the left is reduced to a mere line; at the right it is thicker and so filled with yolk that one can find no line of separation save where the whole has come away from the yolk.

The round-arched type finds a good illustration in fig. 62. There are three points of interest in this section: the presence of vacuoles, which help to separate the right marginal cell from the "*Rand;*" the cell near the center still adherent to the central periblast, and, with its neighbors, having some yolk particles in it; and two pairs of neighboring cells with spindles at right angles to each other. These last illustrate the exceedingly irregular segmentation in the pipefish egg.

Fig. 63 is a nearly central section through a blastoderm intermediate between the normal and the thick-ended types. It is sharply marked off from the outer periblast, which it overhangs on the right. The segmentation cavity is reduced to the interstices between the cells. All along the germ basis, in all the sections, cells are being cut out and the periblast layer left behind. An especially interesting case of this is found in the very center. Some cells show mitotic figures, and in others there are beside the nuclei small solidly staining round bodies of unknown function.

Fig. 64, derived from fig. 59, is a fine example of its type. It is very flat and the segmentation cavity is very much reduced. The periblast, perfectly free from yolk and as distinct below as above, has a layer of cells cut out of it and at the left a nucleus under the marginal cell and clearly derived from it. At one point near the center the periblast is reduced to a mere line. This figure, which is typical for the whole blastoderm, is remarkably like His's (1898) fig. 10 for the brook trout.

STAGE OF TWO-HUNDRED-FIFTY-SIX SURFACE CELLS.

The normal type blastoderm of this stage is shown in fig. 65. The cells lying near the upper surface are considerably smaller than those in the lower parts nearer the periblast. To right and left are furrows with dilatations helping to cut cells out of the periblast, and at the center are cells nearly free from it.

Fig. 66 is plainly a derivative of fig. 63 in its general outline and in the reentrant angles which separate its outer periblast (*o. p.*) from the marginal cells. The periblast is somewhat sunken in the yolk and free from cells throughout the whole blastoderm. The segmentation cavity is, because of this depression, large and is only partly filled with cells. Neighboring sections show the upper surface to be as flat as that in fig. 61.

The third type is shown in fig. 67 from a nearly central section. There is a very noticeable difference in the size of the blastomeres, some being fully three times as large as others. Here again are cells being cut out of the basal periblast. They are in all stages from rounded buds to a completely cut-out cell. Neighboring sections show nuclei in each of these. At the right are two cells connected by a stout protoplasmic bridge.

Fig. 68, Plate X, is a good example of the rounded type. The spacious segmentation cavity is loosely filled with rounded cells. The periblast is throughout the blastoderm in the form of two thick pads in the "*Rand*" region, but in the center it is very thin and obscured with yolk. Nowhere in the whole blastoderm are cells being budded off from it. In the peripheral cells there are, even in this advanced stage, two cases of protoplasmic bridges.

A nearly horizontal section through such a blastoderm as fig. 68 is shown in fig. 69. This is introduced to show the arrangement of cells in horizontal plane. There is here a closer aggregation of cells to the periphery, the inner row being a derivative of the outer, while in the center the cells are more scattering.

Fig. 70 is from a blastoderm intermediate between those from which figs. 65 and 67, Plate IX, are taken. Neighboring sections are more like fig. 65. Some of the outer cells show a tendency to elongate and are somewhat smaller than the interior ones. Both marginal pads are nucleated, and in one a cell wall is cutting downward. While the periblast has cells resting on it and even depressing it, nowhere in the blastoderm is there any evidence that they have been budded off.

STAGE WITH FIVE-HUNDRED-TWELVE CELLS ON THE SURFACE.

Fig. 71, the normal type, is very similar to the preceding figure. Here the cells are pretty uniform in size, and those on the surface are noticeably elongated, some being drawn out in fine thread-like connections—the beginning of the "*Deckschicht*" of the Germans. Some of the nuclei are in process of division by mitosis, but the majority stain solidly. The outer thickenings of the periblast are nucleated, the basal portion is thin, yolky, and totally devoid of either nuclei or cells.

The rounded type is finely shown in fig. 72. The surface cells are slightly flattened and only occasionally pointed, and one on the right is binucleate. The blastomeres are by no means uniform in size, and on the right is a giant cell with a proportionate nucleus. All the nuclei stain solidly. The periblast is very thick, and, while laden with yolk fragments, is fairly distinct below. There are two nuclei in the periblast. One is in a thickening out of which a cell will probably be formed. Near by are cells which seem to have been recently cut out.

Fig. 73 is an excellent illustration of the flat type. The blastomeres are very uniform in size and distribution, and are especially noteworthy for the large number of dividing nuclei, with spindles at all angles. The chief interest, however, centers in the periblast, which is thick and possesses many yolk granules, but is perfectly distinct. In it to the right is a nucleus dividing by mitosis with a spindle considerably longer than those in the blastomeres. On the left the section cuts through a chromatin bundle at right angles to the spindle. At the extreme left is found, for the first time, a nucleus in the outer periblast. The central periblast in this blastoderm is very rich in nuclei dividing by mitosis. A cursory examination showed one vertical and eight horizontal ones. Another blastoderm, of the same lot and stage, contains, in its periblast, thirty-three oblique spindles at all angles from nearly vertical to nearly horizontal, twenty-nine lying horizontally, and seven standing in a vertical position. In all, sixty-nine spindles were counted (none twice). There are a very few solidly staining nuclei, but a great number are cut, as above, through the chromatin masses, and these are not counted. There can be no doubt that the spindles stand in all positions.

The last type of this stage is fig. 74. The cells are not uniform in size, and many are twice as large as the small ones. Most of the nuclei stain solidly, but some contain spindles. Two binucleate cells are present, the one in the periphery being very large. This condition is far from rare in this and later stages. Some thirty cases have been particularly noted. The periblast is very thick, yolky, and distinct. It contains several nuclei, and a cell is either being cut out of or is in process of uniting with the periblast. In other sections similar conditions are found. The reentrant angle, between the outer periblast and the "*Rand*" in this and fig. 73, recalls the like in figs. 63 and 66, Plate IX, and fig. 47, Plate VIII, and in His's figures for the Salmonoids referred to above.

STAGE OF ONE THOUSAND-TWENTY-FOUR SURFACE CELLS.

Fig. 75 represents the normal type and presents several points of interest. The surface cells show a considerable flattening, and adjacent to them are other cells with their bases generally at right angles to the former, making the outer layer in places two cells thick. The inner cells show a tendency to run together in threes and fours. The chief interest, however, centers in the periblast. This is notably free from yolk and is drawn exactly as it appears. Nuclei are scattered very freely throughout its entire extent in all sections, and nearly surround the large vacuole to the right of the center. At the left a large cell, which has recently been cut out of the "*Rand*," is dividing by mitosis. A large number of cells rest on and *indent* the periblast, and are either being cut out of or added to this layer. The close juxtaposition of these cells to nuclei in the periblast would seem to lead to the former conclusion.

The second type is represented in fig. 76, which, judging by the number of cells in the periphery and by their size, is from a blastoderm slightly younger than the preceding. The periblast is sunken deeply into the yolk, and has thus nearly doubled the segmentation cavity, which is sparingly filled with scattered cells. The thick periblast is so obscured with yolk that no nuclei could be found. It is here free from cells, but nearby sections show a condition in this respect like the preceding figure. In the "*Deckschicht*," near the center, is a binucleate cell, while its neighbor has a spindle.

Fig. 77 is from a rounded blastoderm of about the same stage as the preceding. A "*Deckschicht*" can hardly be spoken of here, for the outer cells are nearly all round. The segmentation cavity is reduced to the small interstices between the cells. The greatly thickened periblast is full of large vacuoles, and abounds in nuclei in all the sections, and near the center seems to be budding off cells. In the left "*Rand*" there is a mitotic figure fully twice as large as any in the blastomeres.

No better illustration of the lens-shaped blastoderm so characteristic of late Teleost segmentation than fig. 78 can be given. It probably has been derived from a form like the preceding by the pressure of the cells against the eggshell, causing the periblast to be depressed.

Thus the segmentation cavity has been enlarged and the cells are more scattered than in the preceding. The cells are grouped in twos, threes, and fours. The thick periblast has several nuclei in the resting condition. There is a well defined "epidermic stratum," as the English writers term the outer layer of cells.

Fig. 79 represents the last type of this stage, and need detain us but for a few moments. Its outer cells are flattened and unequal in size, and the interior cells are the largest of all. The periblast is very thick, yolky, and indented from below by large vacuoles. On the left a large cell has been cut out of the "*Rand*," and at the right a cell indents the periblast, while in the center cells seem to be in process of formation from the basal layer. This blastoderm is closely related to that illustrated in section by fig. 74.

Fig. 80 is a horizontal section through some such blastoderm as that illustrated in vertical section in fig. 78, Plate X. It shows the loose arrangement of the interior cells, and the drawn out cells of the "*Deckschicht*." This was broken at several points in the process of sectioning.

LATEST STAGES OF SEGMENTATION.

From this time on it is not profitable and is hardly possible to follow the segmentation, but some figures may be introduced to show the course of development.

Fig. 81, Plate XI, is probably a descendant of a form like fig. 70, Plate X. There is an "epidermic stratum," and the cells are loosely scattered in the large segmentation cavity. The periblast is quite distinct, free from yolk, and has a good many nuclei. Just across the border from one of these nuclei is a cell, in another place a cell lies in a depression in the periblast.

Fig. 82 is another type with "*Deckschicht*," with cells fairly closely crowded in the segmentation cavity, and with a very thin periblast out of which cells are being budded or into which they lose themselves. At one or two places the periblast is reduced to the thickness of a cell wall, and in neighboring sections nuclei abound in it. In the left outer periblast two tripolar spindles are found. These have been noticed occasionally in other sections.

Fig. 83 is the typical Teleost late lens-shaped blastoderm. It closely resembles Fusari's (1890) fig. 9 for *Cristiceps*, and is almost a duplicate of Samassa's (1896) fig. 3 for the salmon in corresponding stages. The depression of the blastoderm into the yolk is probably due to pressure against the eggshell. In the highest part of the epidermic stratum is a very large cell, and in the right "*Rand*" a giant nucleus, which is separated from the neighboring cell by hardly more than the cell wall. At the left a cell has been cut out of the "*Rand*." The thin periblast has resting on it many cells, neither the origin nor the fate of which can safely be passed upon.

EARLY STAGES PREPARATORY TO INVAGINATION.

Fig. 84 is a normal type in which the cells are beginning to move away from the periblast, to crowd together in the upper part of the blastoderm, and to leave a subgerminal cavity (*s. g. c.*) between them and the periblast. The line marked *x* is, in this and the following sections, the lower limit of the cells. The outermost cells of the blastoderm have flattened until they make a very thin skin-like layer. The periblast is comparatively free from yolk granules and is here drawn after nature instead of semi-diagrammatically.

The second type is represented in fig. 85. The cells are densely crowded, the periblast depressed, and the subgerminal cavity (*s. g. c.*) is very large. The periblast is very thick and yolk-laden, and so heavily stained that only one nucleus could be made out.

Fig. 86 illustrates the thick-ended type. In this section the cells are not so closely crowded as in the preceding, but a distinct subgerminal cavity is formed. The very distinct periblast contains many large nuclei, and on the left is separated from the blastoderm by a sharp reentrant angle. A very large binucleate cell is shown, and nearby two others are found. On the left is shown a cell of ordinary size.

Fig. 87 represents the high-arched type like fig. 83, which has begun to flatten out in preparation for the next stage. This flattening is probably responsible for the small subgerminal cavity. The periblast has many large nuclei. Two blastomeres shown indicate the size of the cells at this stage.

LATE STAGES PREPARATORY TO INVAGINATION.

Of these only two will be shown. Fig. 88 is the normal teleost structure for this stage. The cells are all closely crowded into a high-arched band, having a large subgerminal cavity (*s. g. c.*) below. The periblast is here filled with yolk and contains many flattened nuclei. The blastoderm has begun to spread out over the yolk, and the section in fig. 88 is 25 per cent longer than that in fig. 84.

Whether the slight difference in shape of fig. 89 in comparison with fig. 88 is due to contraction caused by the killing fluid or whether it is due to descent from a form like fig. 86 would be hard to decide. Possibly the latter idea is correct. The periblast is filled with yolk fragments, and the nuclei are very much flattened.

VII. THE PERIBLAST.

The origin of this layer, together with many of its peculiarities of structure, has been noted in the descriptions of the plates. It is not my intention to go now into any extended discussion of its formation and fate. However, it will be well to describe briefly the various modes of its formation in other Teleosts, and to show under which of these classes the pipefish egg falls, and finally to give references to a few of the more valuable papers on this subject.

In Teleosts the periblast layer seems to be formed after three types:

(1). In eggs, in which the first furrow cuts through to the yolk, the periblast is formed by a thin protoplasmic sheet extending inward from the "*Rand.*" Henneguy (1888, fig. 63) shows this very plainly for the trout.

(2). In eggs, in which there is no layer of oil drops under the germ-disk, or those in which the protoplasmic mass separates sharply from the yolk, the periblast is formed when the inner ends of the cells in the four and eight celled stages are cut out and lifted from the underlying thin protoplasmic sheet. This is the mode of formation in *Serranus* (Wilson, 1891), *Ctenolabrus* (Agassiz and Whitman, 1885), and *Belone* (Kopsch, 1901).

(3). In eggs in which there is an imperfect separation of germ disk and yolk, or in which there is a layer of oil drops under the blastodisk, the central periblast has a very peculiar mode of origin. Cells are cut out of the protoplasmic disk in successive layers from above downward and the central periblast is the remnant of blastodisk left when this process has ended. The explanation for this is that the protoplasm continues to flow out of the yolk into the germ disk until segmentation has progressed some distance. Kupffer (1868) noticed that the germ disk was not fully formed in a European *Syngnathus* until after the four-celled stage. This formation for the central periblast is described by most workers on the Salmonoids, notably by Zeigler (1882), and Hoffmann (1888), for the salmon, and latest of all by His (1898) for the salmon and trout. Kowalewski (1886) found essentially the same formation in *Carassius* and *Polyacanthus*.

The central periblast nuclei, in types 1 and 2, originate by division of the "*Rand*" nuclei and migrate centralwards in this layer. In Type 3 they are the direct descendants of the segmentation cells.

In *Siphostoma floridæ* there are found the two methods of central periblast formation described in Types 2 and 3 above. In figs. 40, 45, 46, 47, 48, and 52 for the eight and sixteen-celled stages, there is shown a mode of formation for the periblast which negatives the idea that from it there could ever come any "after-segmentation." On the other hand, in figs. 53, 54, 55, 58, 59, 60, 61, and 62, the central periblast is the protoplasmic remnant of the primary germ disk, left after all the blastoderm cells have been cut out of it. It is well to note here that a migration of nuclei into the marginal region and the formation of a "wreath" by the disappearance of cell walls has, because of the opacity of the egg, not been seen in the pipefish. Whether it takes place or not I can not say.

The difficult question, whether, in the egg of the pipefish, cells are budded off from the central periblast and added to the blastomeres, can not here be taken up. However, this would seem to be a legitimate consequence of such a mode of cell formation as that shown in Type 3 above, and apparently finds confirmation in figs. 75, 77, 79, and 82, in which a perfectly definite periblast layer has been formed. If these figures are compared with His's (1898) figs. 10 and 12, this matter will be made clearer.

For a fuller discussion of the origin of the periblast and its nuclei, and of the fate of the latter, the reader is referred to Brook (1887), Kowalewski (1886), Hoffmann (1888), Fusari (1890), Lwoff (1894), Berent (1896), Zeigler (1887 and 1896), His (1898), and Hertwig (1903).

At this point, the work on the development of the pipefish will have to rest. It has been the intention of the writer to carry it further, at least to the closure of the blastopore, and for this purpose the sections have been cut, but the difficulties met with have caused so many delays that it has been impossible to complete it.

The egg of the pipefish is very different from most other teleostean eggs in the form of its segmentation and the dual origin of its periblast, together with the "after-segmentation" of cells therefrom. So marked are these differences that it seems proper to say that the figures in this paper are representative of the sections of a thousand or more eggs, obtained from thirty-three fishes during three summers.

The slides containing the sections from which these figures were drawn have been presented to the U. S. National Museum.

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EXPLANATION OF PLATES.

MAGNIFICATION.

Fig. 1, Plate V, × 38; all other surface views × 73.
All sections × 114.

REFERENCE LETTERS USED IN THE FIGURES.

a–b, *c–d*.	Planes in which were cut sections shown in Plates VII and VIII.
b.	Bud.
c. p.	Central periblast.
d. h.	"*Disque huileux.*"
o.	In fig. 20, furrows not visible in fig. 21.
o. p.	Outer periblast.
p. b.	Protoplasmic bridge.
s. c.	Segmentation cavity.
s. g. c.	Sub-germinal cavity.
v.	Vacuole.
ves.	Vesicle.
x.	Lower limit of cells in figs. 84–88.

PLATE V.

FIG. 1. Egg in shell, blastodisc resting on "*disque huileux*" which covers the upper third of the yolk.
2. Two-celled stage, blastomeres equal.
3. Two-celled stage, blastomeres unequal, vacuole in plane of division.
4. Four-celled stage, regular, segmentation cavity present.
5. Irregular 4-blastomere stage.
6. Four-blastomere stage, reniform, segmentation cavity absent.
7. Normal 8-celled blastoderm.
8. Eight-blastomere stage, slightly irregular.
9. Irregular 8-celled blastoderm.
10. Eight-celled blastoderm formed by equatorial furrow. Cells 4–4.
11. Irregular 8-celled blastoderm with equatorial furrow. Cells 2–6.
12. As above. Two upper cells smaller and shifted to one end.

PLATE VI.

13. Irregular 8-celled blastoderm, with one cell in center.
14. Seven-celled blastoderm, equatorial furrow cutting off 3 upper cells.
15. View of same from below, vertical furrows only visible.
16. Normal 16-celled stage, cells on one level.
17. Normal 16-celled stage, central cells slightly raised.
18. Irregular 16-celled stage. Cells in two layers, blastoderm thicker at lower edge.
19. Irregular 16-celled blastoderm. Cells in two layers, blastoderm highest in center.
20. Irregular 12-celled stage, derived from a form like fig. 14.
21. View of same blastoderm from below, showing small segmentation cavity.

FIG. 22. View from below of a high-arched 16-celled stage, showing 9 cells in the first tier, 6 in the second, and 1 keystone, together with the large segmentation cavity.
23. Nearly normal 32-celled blastoderm.
24. Irregular 27-celled blastoderm. Cells in three tiers, blastoderm thickest at lower edge.
25. Irregular 28-celled stage. Cells in three tiers, blastoderm highest in center.
26. Divided 32-celled blastoderm, 14 cells in smaller, 18 in larger division, both resting on a common protoplasmic basis.
27. Later stage, with 38 cells, showing the growing irregularity of the segmentation.

PLATE VII.

28. Section through germ disc, 5 hours in water. Below the dotted line yolk and protoplasm are mixed.
29. Section through germ disc, 11 hours and 20 minutes in water, showing formation of buds.
30. Section through germ disc 26½ hours in water.
31. Section through germ disc 36–48 hours in pouch.
32. Section through center of 1-celled stage like fig. 1, Plate V.
33. Section through 2-celled stage. Protoplasmic fibrils at right angles to the plane of the furrow.
34. Stage of 2 cells, section through *a–b* of fig. 2.
35. Irregular 2-celled stage, section through *a–b* of fig. 3, showing vesicle at base of furrow.
36. Two-celled stage, furrow taking form of narrow cleft.
37. Furrow of 2-celled stage formed by breaking down of walls of vesicles lying in a vertical series.
38. Horizontal section through 4-blastomere stage of fig. 4.
39. Stage of 8 cells, section through plane *a–b* of fig. 7.
40. Stage of 8 into 16 cells, section through *c–d* of fig. 7.
41. Eight-celled stage, section at right angles to long axis of such stages as figs. 7 and 8, showing protoplasmic bridge.
42. Stage of 8 cells, section in plane *a–b* of fig. 10.

PLATE VIII.

43. Eight-celled stage, section through *a–b* of fig. 12 showing a protoplasmic bridge.
44. Sixteen-celled stage, section in plane *a–b* of fig. 17.
45. Stage of 16 cells, section through plane *a–b* of fig. 16.
46. Stage of 16 cells, section in plane *a–b* of fig. 18.
47. Sixteen-celled stage, section through blastoderm like fig. 19.
48. High-arched 16-celled stage, section through blastoderm like fig. 22, with large segmentation cavity.
49. Stage of 32 cells, section through a blastoderm like fig. 23.
50. Stage of 32 cells, high-arched type, section is through *a–b* of fig. 24.
51. Stage of 32 cells, thick-ended type with large segmentation cavity and thin central periblast.
52. Stage of 32 cells, section through a high-arched blastoderm similar to fig. 25.
53. Thirty-two-celled stage. *No periblast;* two tiers of cells cut out of a solid mass of protoplasm.
54. Divided 32 to 64-celled stage. *No periblast;* basal protoplasm thick, with many vacuoles, and having cell walls cutting down into it.

PLATE IX.

FIG. 55. Divided thick-ended 32 to 64-celled type of blastoderm. The split is here a narrow vertical cleft. Cell walls are pushing into the basal layer which has large vacuoles.

56. Stage of 64 cells. Section through a normal or gently arched type. One cell not yet free from central periblast.

57. Stage of 64 cells. Type with flat surface and abrupt edges. Nuclei are very large and in spireme stage.

58. Same stage. High-arched type. Cells still connected to periblast layer.

59. Section through thick-ended blastoderm of 64-celled stage. No distinct central periblast.

60. Stage of 128 surface cells. Normal type with 7 cells in process of formation from basal layer of protoplasm.

61. Second type of 128-celled stage. Central periblast laden with yolk.

62. High-arched type of this stage. Mitotic spindles stand at all angles to each other, and vacuoles aid in cutting out the cell to right.

63. Fourth type of 128-celled stage. Cells are being cut out of the basal syncytium, the "*Rand*" is separated from the outer periblast by a sharp re-entrant angle. Many of the darkly stained nuclei have beside them solidly stained bodies of unknown function.

64. A section through another thick-ended blastoderm of this stage. There is no central periblast; cells have been cut out of the syncytium.

65. Normal type of 256-celled stage. The nuclei all stain solidly, cells are being cut off from the periblast, the "*Rand*" is nucleated. This is the earliest stage with nuclei in central periblast.

66. Stage of 256 surface cells, second type. "*Rand*" sharply marked off from the outer periblast.

67. Same stage, third type, showing cells in process of formation in the basal syncytium.

PLATE X.

68. High-arched type of this stage, with solidly stained nuclei—periblast wholly free from cells.

69. Horizontal section through blastoderm of same stage as that of which fig. 68 is a vertical section.

70. Vertical section through blastoderm intermediate between figs. 65 and 67.

71. Stage of 512 surface cells, normal type. Surface cells show a notable elongation, some forming "bridges."

72. High-arched type of this stage. The cells are of unequal sizes, the nuclei stain solidly, the periblast is nucleated and in process of budding off cells.

73. Same stage, flat-topped, abrupt-edged type. The "*Rand*" is of peculiar form. At the left a spindle in the periblast is cut through in the chromatin mass while on the right a whole spindle is shown.

74. 512-celled stage, fourth type. "*Rand*" and thick periblast nucleated. Some cells with mitotic figures, but most nuclei stain solidly.

75. Stage of 1,024 cells on surface, normal type. Outer cells flattening to form an epidermic stratum which is at places two-layered. Many nuclei and vacuoles are found in the periblast, out of which a number of cells are being cut.

76. Same stage, second type. The periblast is sunken in the yolk, and the blastomeres only sparingly fill the segmentation cavity thus enlarged. The nuclei are in the spireme stage and a "*Deckschicht*" is present.

77. High-arched type of this stage. The thick periblast is vacuolated and has a giant spindle at the left.

FIG. 78. Section from a blastoderm like fig. 77, but with periblast deeply sunk in the yolk, thus greatly enlarging the segmentation cavity.

79. 1,024-blastomere stage, fourth type. A "*Deckschicht*" is forming and the periblast is giving rise to cells.

PLATE XI.

80. Horizontal section through a blastoderm of the same stage as fig. 78. Epidermic stratum very definite and in part two-layered.

81. Late stage of segmentation. Section from a blastoderm intermediate between the gently arched and the thick-ended types. Blastomeres scattered in the large segmentation cavity caused by the down-sunken periblast.

82. Late segmentation stage, round-arched type. Epidermic layer present. Many cells resting on periblast and probably formed from it. The left outer periblast shows two multipolar spindles.

83. Section through late lens-shaped blastula. The "*Deckschicht*" is two-layered, and the periblast, which has no forming cells, is deeply sunken.

84. Outline section of normal type late blastoderm. The cells have moved upward, forming a compact mass, the lower limit of which is marked x x, and having a large subgerminal cavity. Giant nuclei in periblast.

85. Late blastoderm, second type, showing same structures as fig. 84. Periblas much sunken.

86. Same stage, thick-ended type. Periblast is thin and multinucleate. At large binucleate cell is shown.

87. Same stage and structures as above from a blastoderm like fig. 83. Many resting nuclei in periblast.

88. Normal type blastoderm spreading over yolk preparatory to the beginning of invagination.

89. Section from a blastoderm similar to the above save for a slight variation in shape.

VITA.

Eugene Willis Gudger was born August 10, 1866, at Waynesville, N. C., and received his early education in the public and private schools of that town. He was a student at Emory and Henry College, Virginia, 1883–1885 and 1886–87.

After teaching for three years in public schools, he went to Nashville, Tenn., and in 1891 was graduated with the degree of L. I. from the Peabody Normal College. In 1892 he received the degree of B. S, and in 1893 that of M. S., from the University of Nashville.

He was assistant principal of Orange Street High School, Asheville, N. C., 1893–94, and during the next year was instructor in Asheville Female College. From 1895–1901 he was instructor in sciences in Peabody High School, Little Rock, Ark. He entered Johns Hopkins University in 1901, and from 1902–1904 was student assistant in general biology. His subjects are zoology, botany, and geology.

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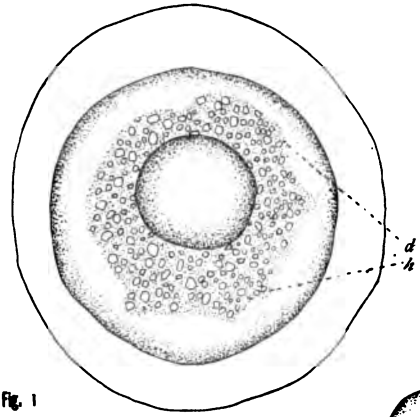


Fig. 1

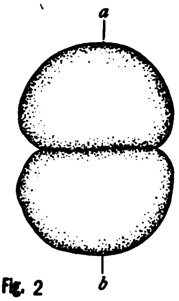


Fig. 2

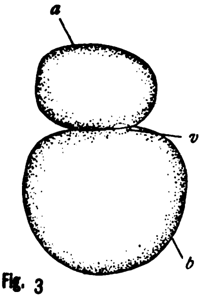
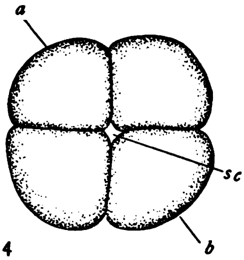


Fig. 3



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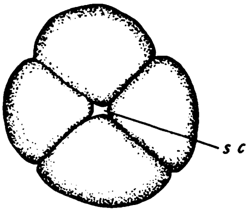
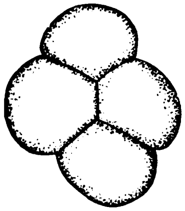


Fig. 5



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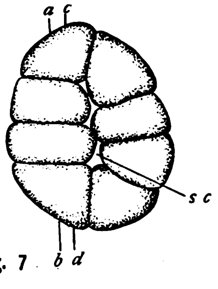
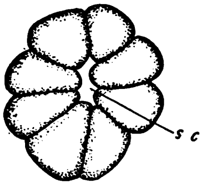


Fig. 7



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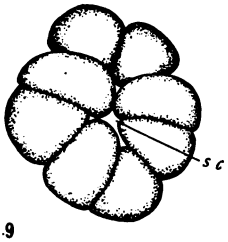
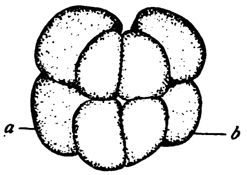


Fig. 9



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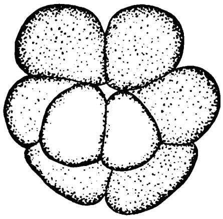


Fig. 11

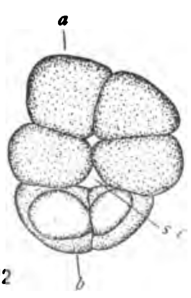


Fig. 12

SEGMENTATION OF PIPEFISH EGGS.

FOR EXPLANATION OF PLATE SEE PAGE 497.

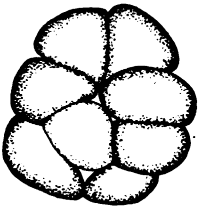
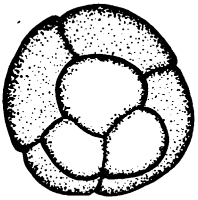


Fig. 13



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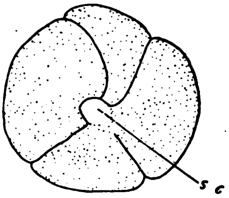


Fig. 15

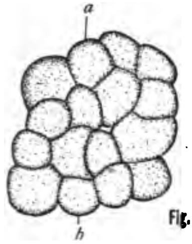
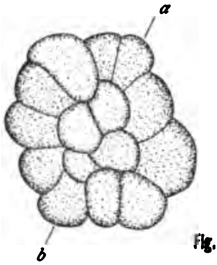


Fig. 16



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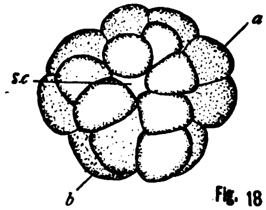

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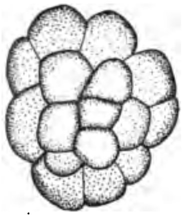
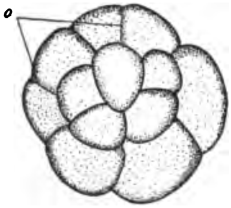


Fig. 19



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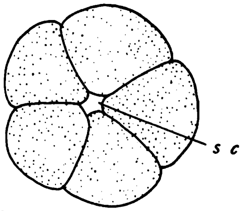


Fig. 21

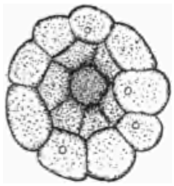
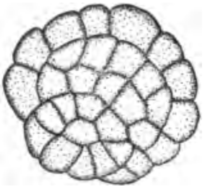


Fig. 22



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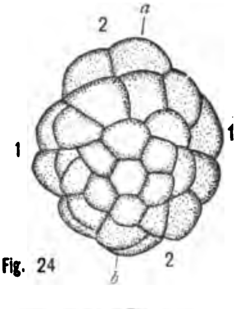


Fig. 24

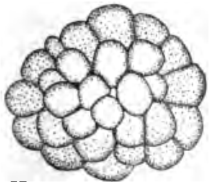


Fig. 25

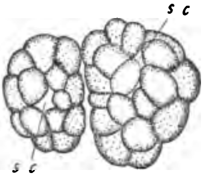


Fig. 26

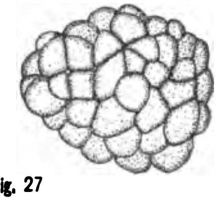
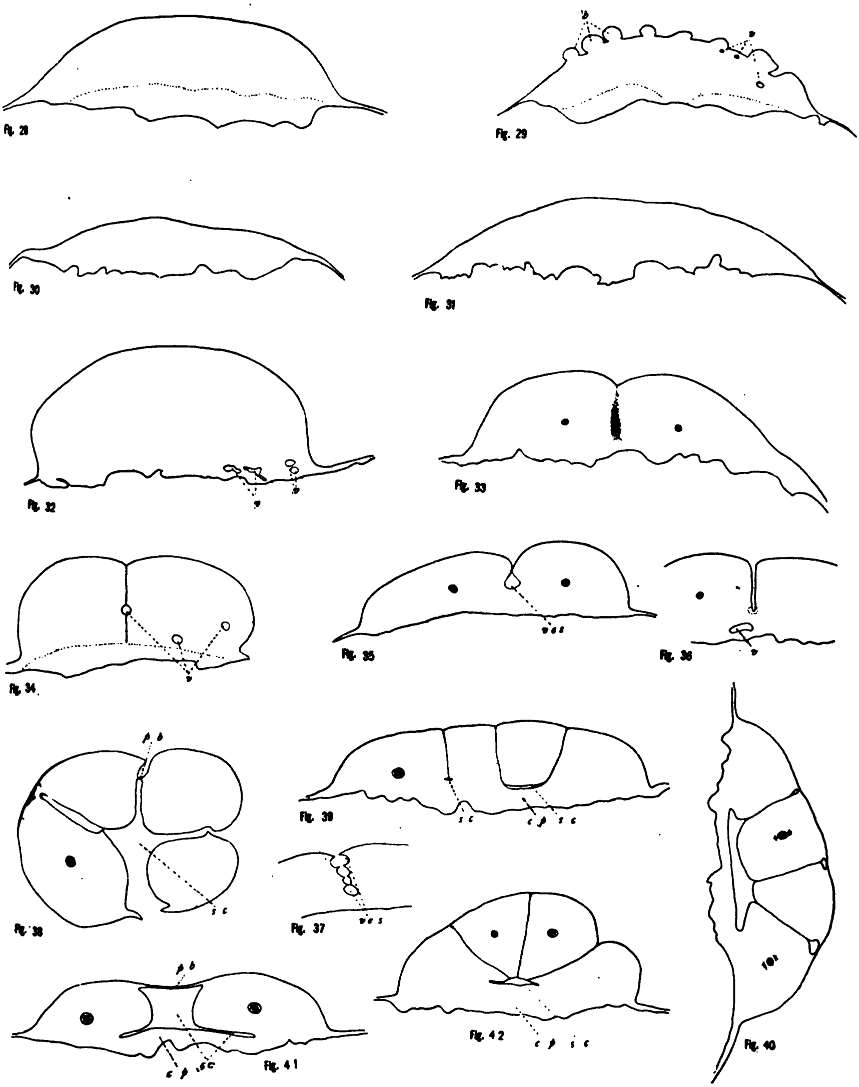


Fig. 27

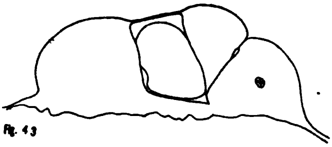
SEGMENTATION OF PIPEFISH EGGS.

FOR EXPLANATION OF PLATE SEE PAGES 497, 498.



SEGMENTATION OF PIPEFISH EGGS.

FOR EXPLANATION OF PLATE SEE PAGE 498.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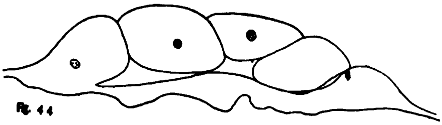
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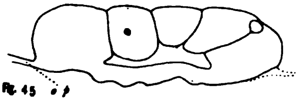

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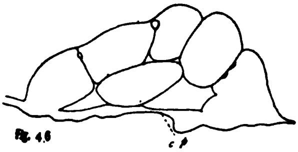

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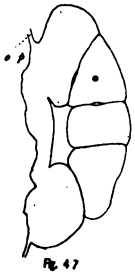

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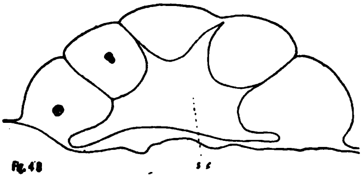

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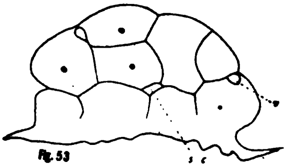

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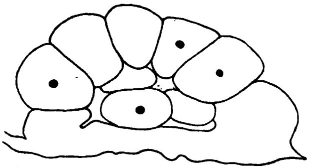
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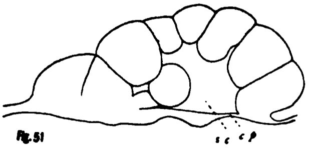

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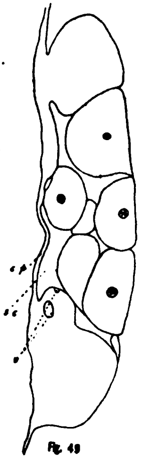

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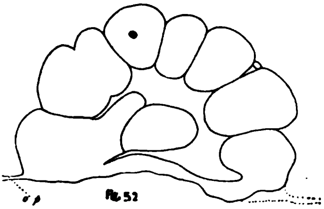

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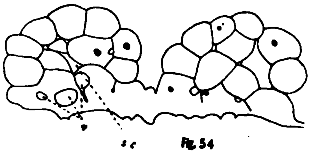
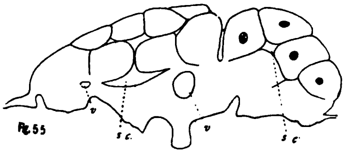


Fig. 54

SEGMENTATION OF PIPEFISH EGGS.

FOR EXPLANATION OF PLATE SEE PAGE 498.



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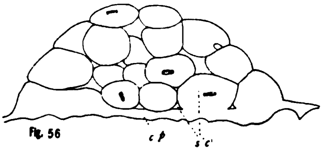

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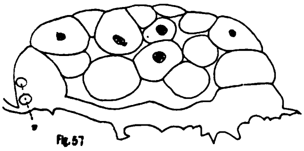

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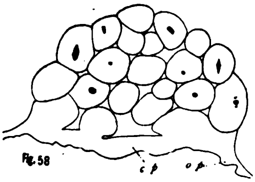

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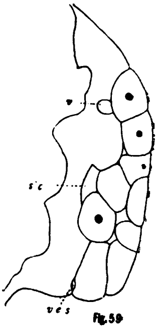

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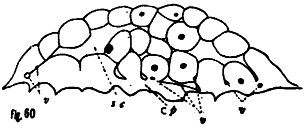

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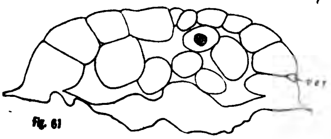

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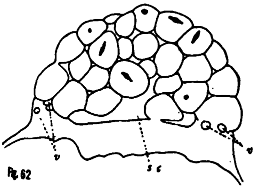

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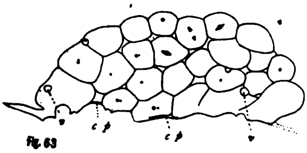

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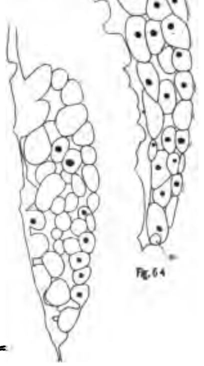

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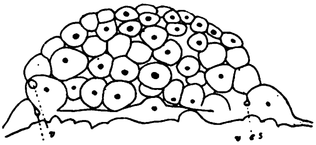

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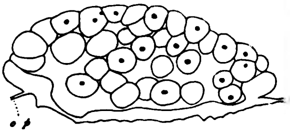
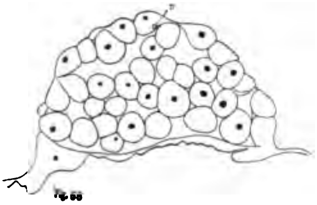


Fig. 66

SEGMENTATION OF PIPEFISH EGGS.

FOR EXPLANATION OF PLATE SEE PAGE 499.



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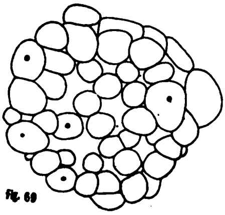

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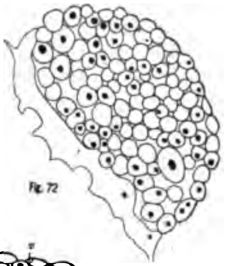

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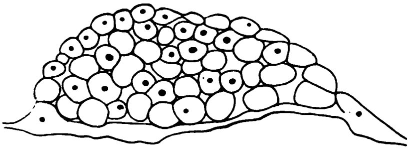

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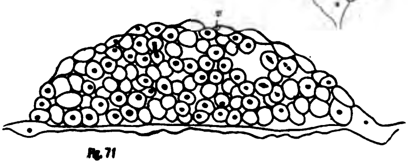

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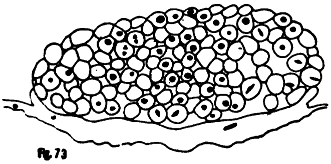

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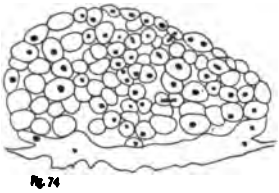

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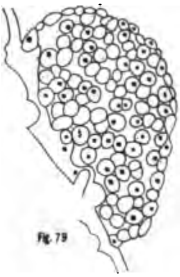

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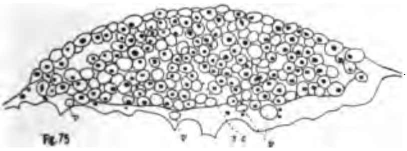

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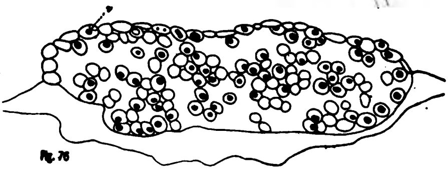

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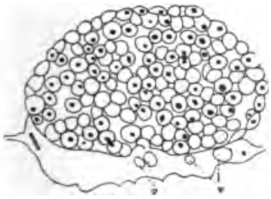

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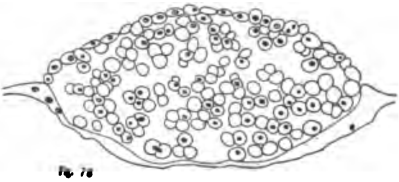
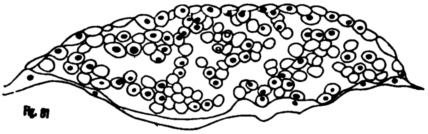


Fig. 78

SEGMENTATION OF PIPEFISH EGGS.

FOR EXPLANATION OF PLATE SEE PAGES 499, 500.



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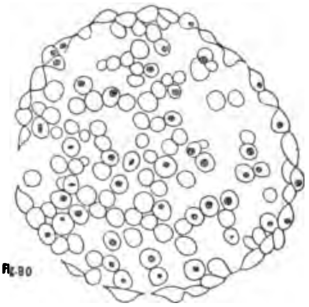

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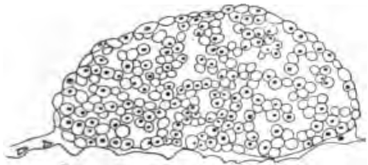

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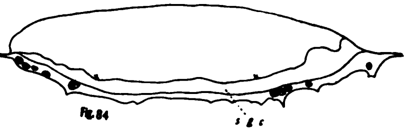

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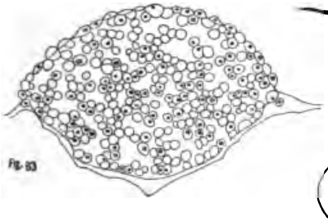

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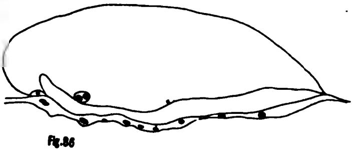

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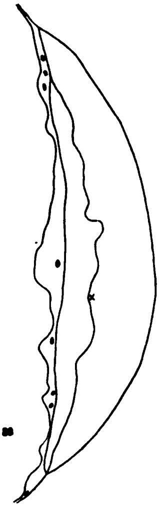

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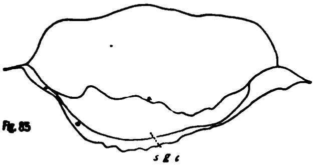

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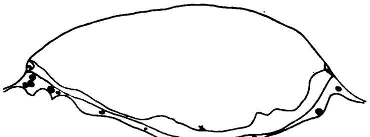

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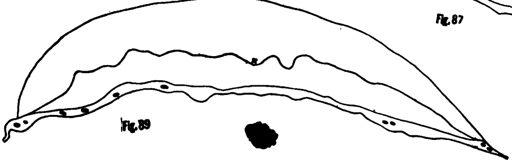

Fig. 89

SEGMENTATION OF PIPEFISH EGGS.

FOR EXPLANATION OF PLATE SEE PAGE 500.